10-02-00   A/Box/seg

UTILITY PATENT APPLICATION TRANSMITTAL (only for continuation and divisional applications under 37 CFR 1.53(b))	Docket No.: P-LJ 4377
	Prior Application Info: Examiner: M. Moran Group/Art Unit: 1631

Address to: COMMISSIONER FOR PATENTS
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Washington, D.C. 20231

23601

PATENT TRADEMARK OFFICE

This is a request for filing a
___ continuation   __X__ divisional

application under 37 CFR 1.53(b), of pending prior application
serial no. **09/258,754**, filed **February 26, 1999**, (list only
immediate prior application).

Title: **METHODS OF IDENTIFYING LUNG HOMING MOLECULES USING
          MEMBRANE DIPEPTIDASE**

Inventor(s)(full name of each inventor):   **Daniel Rajotte
                                            Renata Pasqualini
                                            Erkki Ruoslahti**

No abandonment of, or termination of proceedings, has occurred in
the above-identified prior application.

1.   __x__   An application based on the prior application as filed
             and containing no new matter is enclosed, consisting
             of:
             _1__ page application cover sheet
             _174_ pages of specification (includes claims and
                   abstract)
             _11_ sheets of drawing(s).

2.   __x__   _3__ pages of a copy of the oath or declaration as
                   originally filed on **May 26, 1999,** in prior
                   application (37 CFR 1.63(d)), U.S. serial no.
                   09/258,754, filed **February 26, 1999,** is enclosed.

3.   ____   A signed statement DELETING inventor(s) named in the
             prior application (37 CFR 1.63(d)(2) and 1.33(b)) is
             attached.

4.   _x_   Nucleotide and/or Amino Acid Sequence Submission is
             enclosed:
             __x__ computer readable copy of sequence listing
             __x__ paper copy of sequence listing, pages 1 through
                   134
             __x__ Statement Under § 1.821(f) and (g)
             _____ Request to Use Computer Readable Form of Sequence
                   Listing From Another Application
             _____ Other:

5.   _x_   Incorporation by Reference: The entire disclosure of
             the prior application, from which a copy of the oath or
             declaration is supplied under item no. 2 of this form,
             is considered as being part of the disclosure of the
             accompanying application and is hereby incorporated by
             reference herein.

6.   ____   Enter the unentered amendment previously filed on
             _____ under 37 CFR 1.116 in the
             prior application.

7.   ____   A preliminary amendment is enclosed.

8.   Small entity status:
      a. ____ A small entity statement is enclosed.
      b. _x_ A small entity statement was filed in prior
             application serial no. **09/258,754**, and such status
             is still proper and desired.
      c. ____ is no longer claimed.

9.   _x_   Amend the specification by:
             ___ inserting before the first paragraph on page 1: or
             _x_ deleting the paragraph on page 1 regarding related
                 applications and inserting therefor:

                 This application is a divisional of application
                 serial no. **09/258,754**, filed **February 26, 1999.**
                 (list entire parentage)

10. _x_   Cancel in this application original claims **1 to 4 and
             31 to 40** of the prior application before calculating
             the filing fee.   (At least one original independent
             claims must be retained for filing purposes.)

11.   The prior application is assigned of record to **The Burnham
      Institute.**

12.   The power of attorney in the prior application is to **Cathryn
      Campbell, Reg. No. 31,815.**

13.   ____   A copy of a change in power of attorney or
             authorization of agent as filed in the prior
             application (37 CFR 1.63(d)(4)) is enclosed.

14.   _x_   A return receipt postcard is enclosed.

15.   _x_   A Patent Application Bibliographic Data Sheet is
             enclosed.

Inventors: Rajotte et al.
Docket No.: P-LJ 4377
Page 3

16. Also enclosed:

The filing fee is calculated below:

	NUMBER FILED		NUMBER EXTRA		RATE	FEE
TOTAL CLAIMS	26 - 20	0	6	x	$9 $18	$54
INDEPENDENT CLAIMS	1 - 3	=	0	x	$39 $78	$0
MULTIPLE DEPENDENT CLAIMS _____YES			_X_ NO		$130 $260	$0
BASIC FEE					$345 $690	$345
TOTAL FEE						**$399**

17. _x_   A check in the amount of **$399.00** is enclosed, which
covers the small entity filing fee. A duplicate copy of
this sheet is enclosed.

18. _____   The filing fee is not enclosed. Do not charge our
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19. _x_   The Commissioner is hereby authorized to charge fees
under 37 CFR 1.16 and 1.17 which may be required or
credit any overpayment to Deposit Account No. **03-0370**.
A duplicate copy of this sheet is enclosed.

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Respectfully submitted,

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____ assignee of complete   ____ filed under Sec. 1.34(a)
     interest

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APPLICATION INFORMATION

Title Line One:: METHODS OF IDENTIFYING LUNG HOMING MOLEC
Title Line Two:: ULES USING MEMBRANE DIPEPTIDASE
Total Drawing Sheets:: 11
Formal Drawings?:: Yes
Application Type:: Utility
Docket Number:: P-LJ 4377
Secrecy Order in Parent Appl.?:: No

REPRESENTATIVE INFORMATION

Registration Number One:: 31815
Registration Number Two:: 34949
Registration Number Three:: 44048
Registration Number Four:: 45201
Registration Number Five:: 39200
Registration Number Six:: 41029
Registration Number Seven:: 38444
Registration Number Eight:: 37915

CONTINUITY INFORMATION

This application is a:: DIVISION OF
> Application One:: 09/258,754
  Filing Date:: 02-26-1999

Source:: PrintEFS Version 1.0.1

A P P L I C A T I O N

for

UNITED STATES LETTERS PATENT

on

METHODS OF IDENTIFYING LUNG HOMING MOLECULES USING MEMBRANE DIPEPTIDASE

by

Daniel Rajotte,

Renata Pasqualini

and

Erkki Ruoslahti

Sheets of Drawings: 11

Docket No.: P-LJ 4377

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METHODS OF IDENTIFYING LUNG HOMING MOLECULES
USING MEMBRANE DIPEPTIDASE

This application is a continuation-in-part of
U.S. Application Serial No. 09/042,107, filed
5 March 13, 1998, the entire contents of which is
incorporated herein by reference.

10   **BACKGROUND OF THE INVENTION**

FIELD OF THE INVENTION

The present invention relates generally to
the fields of molecular medicine and drug delivery and,
more specifically, to molecules that home to a specific
15 organ or tissue.

BACKGROUND INFORMATION

Although the effect of a particular pathology
often is manifest throughout the body of the afflicted
person, generally, the underlying pathology may affect
20 only a single organ or tissue. It is rare, however,
that a drug or other treatment will target only the
diseased organ or tissue. More commonly, treatment
results in undesirable side effects due, for example,
to generalized toxic effects throughout the patient's
25 body. It would be desirable to selectively target
organs or tissues, for example, for treatment of
diseases associated with the target organ or tissue.

In particular, targeting of an organ or tissue can be useful for directing the expression of a gene to a certain organ or tissue because incorporation of a foreign gene into nontargeted cells can cause unwanted
5   side effects such as malignant transformation.

Most therapeutic substances are delivered to the target organ or tissue through the circulation. The endothelium, which lines the internal surfaces of blood vessels, is the first cell type encountered by a
10 circulating therapeutic substance in the target organ or tissue. These cells provide a target for selectively directing therapies to an organ or tissue.

Endothelium can have distinct morphologies
15 and biochemical markers in different tissues. The blood vessels of the lymphatic system, for example, express various adhesion proteins that serve to guide lymphocyte homing. For example, endothelial cells present in lymph nodes express a cell surface marker
20 that is a ligand for L-selectin and endothelial cells in Peyer's patch venules express a ligand for the $\alpha_4\beta_7$ integrin. These ligands are involved in specific lymphocyte homing to their respective lymphoid organs. Thus, linking a drug to L-selectin or to the $\alpha_4\beta_7$
25 integrin may provide a means for targeting the drug to diseased lymph nodes or Peyer's patches, respectively, provided that these molecules do not bind to similar ligands present in a significant number of other organs or tissues.

30 Although the homing molecules present in the blood vessels of non-lymphoid tissues have not been clearly defined, certain observations of lymphocyte

circulation suggest that organ and tissue specific
endothelial markers exist. Similarly, the homing or
metastasis of particular types of tumor cells to
specific organs or tissues further suggests that organ
5 and tissue specific markers may exist. Thus, a need
exists to identify molecules that can bind to such
organ or tissue specific markers and, therefore, can
home to the organ or tissue. The present invention
satisfies this need and provides related advantages as
10 well.

SUMMARY OF THE INVENTION

The present invention provides a method of
identifying a membrane dipeptidase (MDP)-binding homing
molecule, which is a molecule that selectively homes to
15 lung endothelium. The method includes the steps of
contacting MDP with one or more molecules; and
determining specific binding of a molecule to the MDP,
where the presence of specific binding identifies the
molecule as a MDP-binding homing molecule that
20 selectively homes to lung endothelium. In a method of
the invention, the MDP can be, for example, in its
natural state or substantially purified and, if
desired, immobilized on a support. The membrane
dipeptidase can be any mammalian MDP, for example,
25 human MDP having SEQ ID NO: 448.

Further provided by the invention is a method
of selectively directing a moiety to lung endothelium
in a subject by administering to the subject a
conjugate containing a moiety linked to a MDP-binding
30 homing molecule that selectively homes to lung
endothelium, whereby the moiety is selectively directed

to lung endothelium in the subject. Such a method can
be useful, for example, for drug targeting to lung. In
a method of the invention, the MDP-binding homing
molecule is identified by contacting membrane
5   dipeptidase (MDP) with one or more molecules; and
determining specific binding of a molecule to the MDP,
where the presence of specific binding identifies the
molecule as a MDP-binding homing molecule that
selectively homes to lung endothelium. A variety of
10   moieties can be selectively directed to lung
endothelium according to a method of the invention. A
moiety useful in the invention can be, for example, a
gene therapy vector or drug.

In one embodiment, the invention provides a
15   method for selectively directing a moiety to lung
endothelium where the MDP-binding homing molecule is a
peptide including the sequence X_1-G-F-E-X_2 (SEQ ID NO:
17), where X_1 and X_2 each is 1 to 10 independently
selected amino acids. Such a MDP-binding homing
20   peptide can include, for example, the sequence
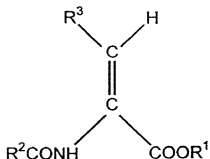CGFECVRQCPERC (SEQ ID NO: 1) or CGFELETC (SEQ ID
NO: 2).

In another embodiment, the invention provides
a method for selectively directing a moiety to lung
25   endothelium where the MDP-binding homing molecule
contains the following Structure 1:

where R^2 and R^3 are hydrocarbon radicals in the range respectively of 3-10 and 1-15 carbon atoms; in either one of these R^2 or R^3 hydrocarbon chains 1-6 hydrogens may be

5   replaced by halogens or a nonterminal methylene may be replaced by oxygen or sulfur, including oxidized forms of the latter; additionally, a terminal hydrogen in R^3 can also be replaced by hydroxyl or thiol, which

10 may be acylated or carbamoylated; or the hydrogen can be replaced by amino, which may be derivatized as in an acylamino, ureido, amidino, quanidino, or alkyl or substituted amino group, including quaternary nitrogen

15 grouping; or, there may be replacement by acid groups such as carboxylic, phosphonic or sulfonic acid groups or esters or amides thereof, or cyano; or combinations thereof, such as a terminal amino acid grouping; and R^1

20 is hydrogen or lower alkyl (C_{1-6}) or dialkylaminoalkyl, or a pharmaceutically acceptable cation. Such an MDP-binding homing molecule for reducing or preventing lung metastasis can be, for example, 7-(L-2-amino-

25 2-carboxyethylthio)-2-(2,2-dimethylcyclopropane carboxamido)-2-heptenoic acid, also known as cilastatin.

        An MDP-binding homing molecule can be, for example, a compound having Structure 1

30 in which R^2 is branched alkyl or cycloalkyl with a limitation that the carbon adjacent to the carbonyl cannot be tertiary.

An MDP-binding homing molecule also can be, for example, a compound having Structure 1 in which R^2 is branched alkyl or cycloalkyl with a limitation that the carbon
5 adjacent to the carbonyl cannot be tertiary, and in which R^3 is n-alkyl (1-9 carbons) or n-alkyl (1-9 carbons) having a terminal substituent which is a quaternary nitrogen, amine derivative or amino acid derived group.
10 An MDP-binding homing molecule can be, for example, a compound having Structure 1 in which R^2 is 2,2-dimethylcyclopropyl or 2,2-dichlorocyclopropyl and in which R^3 is a hydrocarbon chain of 3 to 7 carbon atoms
15 without a terminal substituent or having a terminal substituent which is trimethylammonium, amidino, guanidino or 2-amino-2-carboethylthio.

Exemplary MDP-binding homing
20 molecules having Structure 1 useful in the invention include the following: Z-2-(2,2-dimethylcyclopropane carboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt; Z-2-(2,2-dichlorocyclo
25 propanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt; Z-2-(2,2-dimethylcyclopropane carboxamido)-8-guanidino-2-octenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-
30 guanidino-2-octenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-ureido-2-octenoic acid; Z-8-(1-2-amino-2-carboxy ethylthio)-2-(2,2-dimethylcyclopropane

carboxamido)-2-octenoic acid; Z-2-(2,2-
dimethylcyclopropane carboxamido)-2-octenoic
acid (racemic and dextrorotatory forms); Z-2-
(2,2-dichloro cyclopropanecarboxamido)-2-
5   octenoic acid; 7-(L-2-amino-2-
carboxyethylthio) -2-(2,2-dimethylcyclopropane
carboxamido)-2-heptenoic acid; and 6-(L-2-
amino-2-carboxyethylthio)-2-(2,2-
dimethylcyclopropane carboxamido)-2-hexenoic
10   acid.

The present invention also provides a method
of reducing or preventing lung metastasis in a subject
having cancer by administering to the subject a
membrane dipeptidase (MDP)-binding homing molecule. In
15   a preferred embodiment, an MDP-binding homing molecule
is a lung homing peptide including the sequence
X_1-G-F-E-X_2 (SEQ ID NO: 17), where X_1 and X_2 each is 1
to 10 independently selected amino acids, such as a
peptide including the sequence CGFECVRQCPERC (SEQ ID
20   NO: 1) or CGFELETC (SEQ ID NO: 2).

In another preferred embodiment, an
MDP-binding homing molecule is a molecule containing
Structure 1, described hereinabove. Such an
MDP-binding homing molecule can be, for example,
25   7-(L-2-amino-2-carboxyethylthio)-2-(2,2-
dimethylcyclopropane carboxamido)-2-heptenoic acid,
commonly known as cilastatin.

In one embodiment, an MDP-binding homing
molecule useful in the invention is an MDP inhibitor.
30   Such an MDP inhibitor can exhibit, for example, a Ki
against MDP of 1000 nM or less. In other embodiments,

an MDP inhibitor useful in reducing or preventing lung
metastasis exhibits a Ki against MDP of 100 nM or less
or a Ki against MDP of 1 nM or less.

5   The present invention also provides a method
of reducing or preventing lung metastasis in a subject
having cancer by administering to the subject a MDP
negative regulatory agent. A MDP negative regulatory
agent useful in the invention can be, for example, a
soluble MDP polypeptide or an antibody that selectively
10   reacts with MDP.

       Further provided herein are methods of
reducing or preventing cell homing to lung endothelium
in a subject by administering to the subject a MDP
negative regulatory agent. A MDP negative regulatory
15   agent useful for reducing or preventing cell homing to
lung endothelium can be, for example, a soluble MDP
polypeptide or an antibody that selectively reacts with
MDP.

       The present invention also provides a method
20   of identifying a molecule that reduces or prevents lung
metastasis by contacting membrane dipeptidase (MDP)
with one or more molecules; and determining MDP
activity in the presence of the molecule as compared to
a control value, where diminished MDP activity in the
25   presence of the molecule identifies the molecule as a
molecule that reduces or prevents lung metastasis. The
membrane dipeptidase can be, for example, substantially
purified. MDP activity can be determined, for example,
by release of D-Phe from Gly-D-Phe.

30

In one embodiment, the invention provides a
method of identifying a molecule that reduces or
prevents lung metastasis by contacting MDP with one or
more molecules; determining MDP activity in the
5   presence of the molecule as compared to a control
value; administering the molecule to a subject having
cancer; and assaying lung metastasis in the subject as
compared to a control level of metastasis, where
diminished MDP activity in the presence of the molecule
10   identifies the molecule as a molecule that reduces or
prevents lung metastasis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the results of three rounds of
15   *in vivo* panning of a CX$_6$C (SEQ ID NO: 26) library for
identifying molecules that home to lung. Phage
recovered from the lung five minutes after injection of
10^{10} transducing units into the tail vein of mice were
amplified and reinjected in two consecutive rounds.
20   The number of phage recovered per gram of lung, kidney
or brain is indicated for each round, with bars
representing standard error of the mean from triplicate
platings.

Figures 2A to 2D show the selectivity of
25   phage displaying a lung (Figures 2A and 2B), skin
(Figure 2C) or pancreas (Figure 2D) homing peptides.
Selected phage expressing peptides that home to lung
(Figure 2A, CGFECVRQCPERC, SEQ ID NO: 1, "GFE-1";
Figure 2B, CGFELETC, SEQ ID NO: 2, "GFE-2"); skin
30   (Figure 2C, CVALCREACGEGC, SEQ ID NO: 3) or pancreas
(Figure 2D, SWCEPGWCR, SEQ ID NO: 4) were individually

amplified, injected into mice and recovered
(Example I). The amount of phage displaying a selected
peptide that was recovered per gram of lung, skin or
pancreas or control kidney or brain was determined.
5   The amount of unselected (control) phage recovered from
lung, skin, pancreas, kidney or brain also was
determined. Bars indicate standard error of the mean
from triplicate platings.

Figures 3A and 3B show the effect of
10   coadministration of glutathione S-transferase- (GST)-
fusion proteins on the homing of phage displaying lung
or skin homing peptides.

In Figure 3A, 100 μg or 500 μg of
GST-CGFECVRQCPERC (SEQ ID NO: 1, "GFE-1") or 500 μg GST
15   was coinjected into mice with 10^9 transducing units of
the individually amplified phage displaying the lung
homing sequence CGFECVRQCPERC (SEQ ID NO: 1). The
recovery of phage after 5 minutes of circulation from
lung and kidney was determined, with bars indicating
20   standard error of the mean from triplicate platings.

In Figure 3B, 10^9 transducing units of
individually amplified phage displaying either the lung
homing peptides CGFECVRQCPERC (SEQ ID NO: 1, "GFE-1")
or CGFELETC (SEQ ID NO: 2, "GFE-2") or the skin homing
25   peptide CVALCREACGEGC (SEQ ID NO: 3) were coinjected
into mice with 500 μg of the cognate GST-fusion
peptide. Control mice (not shown) were injected with
the selected phage and 500 μg of GST. The percentage
of inhibition of selected phage homing to lung or skin
30   in the presence of the cognate GST-fusion peptide
compared to GST is shown.

Figure 4 shows that a 55 kDa cell surface protein from lung extracts specifically binds to a GFE-1 peptide affinity column. An *in vivo* biotinylated lung extract was fractionated on a CGFECVRQCPERC (SEQ ID NO: 1; GFE-1) peptide column or on a control peptide column (GRGESP; SEQ ID NO: 442), washed, and eluted with GFE-1 peptide. Aliquots (30 µl) from the wash fraction ("-"), GFE-1 peptide elution, and 8M urea elution were resolved by SDS-PAGE under reducing conditions. Molecular weight markers (in kDa) are indicated on the right side of each panel.

Figure 5 shows a time course of the fluorimetric detection of D-phenylalanine (D-Phe) produced by hydrolysis of Gly-D-Phe. Samples from the wash fraction (○) and the CGFECVRQCPERC (SEQ ID NO: 1; GFE-1) peptide eluate (●) from a GFE-1 peptide affinity column were assayed for MDP activity with a Gly-D-Phe substrate. The time-dependent conversion of D-Phe into a fluorescent compound is shown.

Figure 6 shows binding of phage bearing peptide CGFECVRQCPERC (SEQ ID NO: 1; GFE-1) or CGFELETC (SEQ ID NO: 2; GFE-2) to COS-1 cells expressing membrane dipeptidase (MDP).

In Figure 6A, COS-1 cells were transfected with the MDP expression vector or vector alone. Two days after the transfection, cell extracts were prepared and analyzed for MDP activity.

In Figure 6B, COS-1 cells transfected with either the MDP expression vector or control empty vector were subjected to a binding assay in the

presence of equal amounts of either control phage
(fd-tet) or phage bearing the skin-homing peptide
CVALCREACGEGC (SEQ ID NO: 3; "shp-1"), CGFECVRQCPERC
(SEQ ID NO: 1; GFE-1) peptide, or CGFELETC (SEQ ID
5 NO: 2; GFE-2) peptide. In each case, the total number
of phage transducing units rescued from these cells is
shown. Error bars indicate standard deviation of the
mean from triplicate platings.

Figure 7 shows inhibition of MDP activity by
10 the GFE-1 peptide CGFECVRQCPERC (SEQ ID NO: 1).
Extracts from MDP-expressing COS-1 cells were assayed
for MDP activity in the presence of increasing
concentrations of CGFECVRQCPERC (SEQ ID NO: 1; GFE-1)
(●) or CARAC control peptide (SEQ ID NO: 443), shown as
15 (○).

Figure 8 shows that GFE-1 (SEQ ID NO: 1)
inhibits lung metastasis of human melanoma cells. Lung
weight is shown for mice five weeks after injection
with 10^5 C8161 human melanoma cells alone ("vehicle");
20 10^5 C8161 cells coadministered with 250 μg GFE-1
peptide SEQ ID NO: 1 ("GFE-1 peptide"); or 10^5 C8161
cells coadministered with 250 μg CARAC peptide SEQ ID
NO: 443 ("control peptide").

Figure 9 shows an alignment of the predicted
25 amino acid sequences of membrane dipeptidase from five
species. The amino acid sequences of the human ("HUM;"
SEQ ID NO: 448); pig ("PIG;" SEQ ID NO: 449); rat
("RAT;" SEQ ID NO: 450); and mouse ("MOU;" SEQ ID NO:
451) MDPs are aligned together with the sequence of
30 rabbit MDP ("RAB;" SEQ ID NO: 452). Asterisks indicate
N-linked glycosylation sites in human MDP. The boxed

residues Glu^{125} and His^{219} are essential for activity
(Adachi et al., Biochim. Biophys. Acta 1163:42-48
(1993); Keynan et al., FEBS Letters 349:50-54 (1994)).
Underlined residues (-1 to -16) represent the signal
5   peptide. The boxed residues at the C-terminus indicate
the hydrophobic signal that is replaced by a
glycosyl-phosphatidylinositol (GPI) anchor in the
mature protein. The site of GPI anchor addition is
indicated by an arrow.

10   **DETAILED DESCRIPTION OF THE INVENTION**

            The present invention provides organ and
tissue homing molecules and methods of using these
molecules to target a moiety to a selected organ or
tissue. The molecules of the invention, which were
15 identified essentially by the method of *in vivo* panning
(U.S. Patent No. 5,622,699, issued April 22, 1997,
which is incorporated herein by reference), include
peptides that home to various normal organs or tissues,
including lung, skin, pancreas, retina, prostate,
20 ovary, lymph node, adrenal gland, liver or gut, and to
organs bearing tumors, including to lung bearing lung
tumors and to pancreas bearing a pancreatic tumor. For
example, the invention provides lung homing peptides,
including the peptides CGFECVRQCPERC (SEQ ID NO: 1) and
25 CGFELETC (SEQ ID NO: 2), each of which contains a
tripeptide GFE motif, and the peptide GIGEVEVC (SEQ ID
NO: 8). The invention also provides skin homing
peptides such as the peptide CVALCREACGEGC (SEQ ID
NO: 3); pancreas homing peptides such as the peptide
30 SWCEPGWCR (SEQ ID NO: 4) and retina homing peptides
such as the peptides CSCFRDVCC (SEQ ID NO: 5) and
CRDVVSVIC (SEQ ID NO: 6), each of which contains a

tripeptide RDV motif. Examples of peptides that home
to prostate, ovary, lymph node, adrenal gland, liver
and gut are also provided (see Tables 2 to 11). It
should be recognized that motifs common to particular
5 organ homing peptides can be identified by simple
inspection of the peptides. For example, inspection of
Table 9 reveals that the peptides AGCSVTVCG (SEQ ID
NO: 315) and AGCVQSQCY (SEQ ID NO: 370) share an AGC
motif; the peptides LECRRWRCD (SEQ ID NO: 328) and
10 LECVANLCT (SEQ ID NO: 337) share an LEC motif; and the
peptides SECAYRACS (SEQ ID NO: 319) and SECYTGSCP (SEQ
ID NO: 375) share an SEC motif. In addition, several
of these peptides were isolated more than one time (see
asterisks in Table 9), indicating that such motifs are
15 relevant to the ability of the peptides to selectively
home. Peptides comprising the particular motifs
disclosed herein, as well as other motifs identifiable
by inspection of the disclosed peptides, are considered
within the claimed invention, provided that the motif
20 is not an RGD motif.

        The homing molecules of the invention are
useful for targeting a moiety to a particular organ or
tissue. Thus, the invention provides conjugates,
comprising an organ homing molecule linked to a moiety.
25 Such moieties can be a therapeutic agent such as a
virus; a viral gene therapy vector; a drug; a
detectable or imaging agent such as a radionuclide; or
a tag such as biotin. As disclosed herein, such organ
homing molecules of the invention, particularly
30 conjugates of the invention, can be used to detect or
visualize a selected organ or tissue or to diagnose or
treat a pathology in a selected organ or tissue. An
organ homing molecule of the invention also can be used

to isolate the target molecule that is expressed in the selected organ or tissue and binds the organ homing molecule. For convenience, a molecule of the invention that homes to a selected organ or tissue is referred to

5 as an "organ homing molecule."

As used herein, the term "molecule" is used broadly to mean an organic compound having at least one reactive group that can be varied by substituting one or more different groups. An organic molecule can be a

10 drug; a nucleic acid molecule, including RNA or DNA; a peptide; a variant or modified peptide or a peptide mimetic; a protein or a fragment thereof; an oligosaccharide; a lipid; a glycolipid; or a lipoprotein.

15 An organic molecule can be a naturally occurring molecule, which can be a product of nature in that the groups comprising the organic molecule and the bonds linking the groups are produced by biological processes. For example, a naturally occurring organic

20 molecule can be an RNA molecule or a fragment thereof, which can be isolated from a cell or expressed from a recombinant nucleic acid molecule. Similarly, a peptide is considered a naturally occurring organic molecule, even if it is produced by chemical synthesis,

25 since the amino acid groups and bonds linking the groups can be produced by normal biological processes and the peptide, itself, can be produced in a cell due, for example, to proteolytic degradation of a protein containing the peptide.

30 An organic molecule also can be a nonnaturally occurring molecule. Such molecules have

chemical groups or bonds that are not normally produced
by biological processes. For example, a nucleic acid
sequence containing nonnaturally occurring nucleoside
analogs or phosphorothioate bonds that link the
5 nucleotides and protect against degradation by
nucleases are examples of nonnaturally occurring
molecules. A ribonucleotide containing a 2-methyl
group, instead of the normal hydroxyl group, bonded to
the 2'-carbon atom of ribose residues, is an example of
10 a non-naturally occurring RNA molecule that is
resistant to enzymatic and chemical degradation. Other
examples of nonnaturally occurring organic molecules
include RNA containing 2'-aminopyrimidines, such RNA
being 1000x more stable in human serum and urine as
15 compared to naturally occurring RNA (see Lin et al.,
Nucl. Acids Res., 22:5229-5234 (1994); and Jellinek
et al., Biochemistry, 34:11363-11372 (1995), each of
which is incorporated herein by reference).

For convenience, the term "peptide" is used
20 broadly herein to mean peptides, polypeptides, proteins
and fragments of proteins. Other molecules useful in
the invention include peptoids, peptidomimetics and the
like. With respect to the organ or tissue homing
peptides of the invention, peptidomimetics, which
25 include chemically modified peptides, peptide-like
molecules containing nonnaturally occurring amino
acids, peptoids and the like, have the binding activity
of an organ homing peptide upon which the
peptidomimetic is derived (see, for example, "Burger's
30 Medicinal Chemistry and Drug Discovery" 5th ed.,
vols. 1 to 3 (ed. M.E. Wolff; Wiley Interscience 1995),
which is incorporated herein by reference).
Peptidomimetics provide various advantages over a

peptide, including that a peptidomimetic can be stable when administered to a subject, for example, during passage through the digestive tract and, therefore, useful for oral administration.

5   Methods for identifying a peptidomimetic are well known in the art and include, for example, the screening of databases that contain libraries of potential peptidomimetics. For example, the Cambridge Structural Database contains a collection of greater than 300,000 compounds that have known crystal structures (Allen et al., Acta Crystallogr. Section B, 35:2331 (1979)). This structural depository is continually updated as new crystal structures are determined and can be screened for compounds having suitable shapes, for example, the same shape as an organ or tissue homing molecule, as well as potential geometrical and chemical complementarity to a target molecule bound by an organ or tissue homing peptide. Where no crystal structure of a homing peptide or a target molecule, which binds an organ or tissue homing molecule, is available, a structure can be generated using, for example, the program CONCORD (Rusinko et al., J. Chem. Inf. Comput. Sci. 29:251 (1989)). Another database, the Available Chemicals Directory (Molecular Design Limited, Informations Systems; San Leandro CA), contains about 100,000 compounds that are commercially available and also can be searched to identify potential peptidomimetics of an organ or tissue homing molecule.

30   The term "nucleic acid molecule" also is used broadly to mean any polymer of two or more nucleotides, which are linked by a covalent bond such as a

phosphodiester bond, a thioester bond, or any of various other bonds known in the art as useful and effective for linking nucleotides. Such nucleic acid molecules can be linear, circular or supercoiled, and

5   can be single stranded or double stranded DNA or RNA or can be a DNA/RNA hybrid.

As used herein, the term "library" means a collection of molecules. A library can contain a few or a large number of different molecules, varying from

10   about two to about 10^{15} molecules or more. The chemical structure of the molecules of a library can be related to each other or be diverse. If desired, the molecules constituting the library can be linked to a common or unique tag, which can facilitate recovery and/or

15   identification of the molecule.

Methods for preparing libraries containing diverse populations of various types of molecules such as peptides, peptoids and peptidomimetics are well known in the art and various libraries are commercially

20   available (see, for example, Ecker and Crooke, Biotechnology 13:351-360 (1995), and Blondelle et al., Trends Anal. Chem. 14:83-92 (1995), and the references cited therein, each of which is incorporated herein by reference; see, also, Goodman and Ro, Peptidomimetics

25   for Drug Design, in "Burger's Medicinal Chemistry and Drug Discovery" Vol. 1 (ed. M.E. Wolff; John Wiley & Sons 1995), pages 803-861, and Gordon et al., J. Med. Chem. 37:1385-1401 (1994), each of which is incorporated herein by reference). Where a molecule is

30   a peptide, protein or fragment thereof, the molecule can be produced *in vitro* directly or can be expressed from a nucleic acid, which can be produced *in vitro*.

Methods of synthetic peptide and nucleic acid chemistry are well known in the art.

A library of peptide molecules also can be produced, for example, by constructing a cDNA
5   expression library from mRNA collected from a cell, tissue, organ or organism of interest. Methods for producing such libraries are well known in the art (see, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory
10 Press 1989), which is incorporated herein by reference). Preferably, a peptide encoded by the cDNA is expressed on the surface of a cell or a virus containing the cDNA. For example, cDNA can be cloned into a phage vector such as fuse 5 (Example I),
15 wherein, upon expression, the encoded peptide is expressed as a fusion protein on the surface of the phage.

In addition, a library of molecules can be a library of nucleic acid molecules, which can be DNA,
20 RNA or analogs thereof. For example, a cDNA library can be constructed from mRNA collected from a cell, tissue, organ or organism of interest, or by collecting genomic DNA, which can be treated to produce appropriately sized fragments using restriction
25 endonucleases or methods that randomly fragment genomic DNA. A library comprising RNA molecules also can be constructed by collecting RNA from cells or by synthesizing the RNA molecules chemically. Diverse libraries of nucleic acid molecules can be made using
30 solid phase synthesis, which facilitates the production of randomized regions in the molecules. If desired, the randomization can be biased to produce a library of

nucleic acid molecules containing particular
percentages of one or more nucleotides at a position in
the molecule (U.S. Patent No.: 5,270,163, issued
December 14, 1993, which is incorporated herein by
5   reference).

          If desired, the nucleic acid molecules can be
nucleic acid analogs that are less susceptible to
degradation by nucleases. For example, RNA molecules
containing 2'-0-methylpurine substitutions on the
10   ribose residues and short phosphorothioate caps at the
3'- and 5'-ends exhibit enhanced resistance to
nucleases (Green et al., Chem. Biol., 2:683-695 (1995),
which is incorporated herein by reference). Similarly,
RNA containing 2'-amino- 2'-deoxypyrimidines or
15   2'-fluro- 2'-deoxypyrimidines is less susceptible to
nuclease activity (Pagratis et al., Nature
Biotechnol., 15:68-73 (1997), which is incorporated
herein by reference). Furthermore, L-RNA, which is a
stereoisomer of naturally occurring D-RNA, is resistant
20   to nuclease activity (Nolte et al., Nature Biotechnol.,
14:1116-1119 (1996); Klobmann et al., Nature
Biotechnol., 14:1112-1115 (1996); each of which is
incorporated herein by reference). Such RNA molecules
and methods of producing them are well known and
25   routine (see Eaton and Piekern, Ann. Rev. Biochem.,
64:837-863 (1995), which is incorporated herein by
reference). DNA molecules containing phosphorothioate
linked oligodeoxynucleotides are nuclease resistant
(Reed et al., Cancer Res. 50:6565-6570 (1990), which is
30   incorporated herein by reference). Phosphorothioate-3'
hydroxypropylamine modification of the phosphodiester
bond also reduces the susceptibility of a DNA molecule
to nuclease degradation (see Tam et al., Nucl. Acids

Res., 22:977-986 (1994), which is incorporated herein
by reference). If desired, the diversity of a DNA
library can be enhanced by replacing thymidine with
5-(1-pentynyl)-2'-deoxoridine (Latham et al., Nucl.
5   Acids Res. 22:2817-2822 (1994), which is incorporated
herein by reference). Such modified nucleic acid
molecules can be useful for the manufacture of a
library or for the purpose of being a tag, which is
described later below.

10   As disclosed herein, *in vivo* panning for the
purpose of identifying an organ or tissue homing
molecule comprises administering a library to a
subject, collecting an organ or tissue sample and
identifying an organ or tissue homing molecule using
15   various methods well known in the art. Generally, the
presence of an organ or tissue homing molecule in a
collected organ or tissue is identified based on one or
more characteristics common to the molecules present in
the library, then the structure of a particular organ
20   or tissue homing molecule can be determined.

          A highly sensitive detection method such as
mass spectrometry (MS), either alone or in combination
with a method such as gas chromatography (GC), can be
used to identify homing molecules that are closely
25   related even when present in small amounts in a
selected organ or tissue. For example, GC in
combination with MS was used to identify two major and
four minor lidocaine metabolites following lidocaine
injection into rats and the analysis of urine (Coutts
30   et al., J. Chromotogr. 421:267-280 (1987), which is
incorporated herein by reference). Similarly, where a
library comprises diverse molecules based generally on

the structure of an organic molecule such as a drug, an organ or tissue homing molecule can be identified by determining the presence of a parent peak for the particular molecule.

5   If desired, the selected organ or tissue can be processed using a method such as HPLC, which can be used to obtain an enriched fraction of molecules having a defined range of molecular weights or polarity or the like from a complex mixture. The enriched fraction of

10   molecules then can be further analyzed for the purposes of identifying organ or tissue homing molecules. For example, HPLC coupled with GC and MS were used to identify seven metabolites of a vitamin D analogue after injection of dihydrotachysterol 3 into a rat and

15   fractionation of an isolated perfused kidney (Porteous et al., Biomed. Environ. Mass Spectrum 16:87-92 (1988), which is incorporated herein by reference). Conditions for HPLC will depend on the structure of the particular molecule and can be optimized by those skilled in the

20   art based on knowledge of the molecule.

          The organ homing molecules present in a collected sample of organ or tissue can be recovered from the sample by incubation in a solution having a defined salt concentration and temperature. Selective

25   extraction also can be used to obtain different fractions of organic molecules by sequentially incubating a collected sample in one or more solutions. Such solutions can have a different salt concentration or can effect extraction of an organic homing molecule

30   at a particular temperature. The resulting eluates from the collected sample can be collected separately or can be pooled into one or more fractions and the

organ homing molecules can be detected and identified.
Similarly, methods for bulk removal of potentially
interfering cellular materials such as DNA, RNA,
proteins, lipids or carbohydrates are well known in the

5 art. Such methods can be used to enrich for the
particular organ homing molecule from potentially
contaminating materials in the collected sample and to
increase the sensitivity of detecting the molecule.

Ease of identification of an organ or tissue

10 homing molecule, particularly an untagged molecule,
depends upon various factors, including the presence of
potentially contaminating background cellular material.
For example, where the homing molecule is an untagged
peptide, a larger number must home to the organ or

15 tissue in order to identify the specific peptides over
the background of cellular protein. In contrast, a
much smaller amount of an untagged homing molecule such
as a drug is identifiable because such molecules
normally are generally absent from or present in very

20 small numbers in the body. In this situation, a highly
sensitive method such as MS can be used to identify an
organ homing molecule. The skilled artisan will
recognize that the method of identifying a molecule
will depend, in part, on the structure of the

25 particular molecule.

As disclosed herein, a sufficient number of
molecules selectively home to a selected organ or
tissue during *in vivo* panning such that the molecules
readily can be identified. For example, peptides that

30 were identified two or more times in a particular
collected organ (see Table 1). For example, of forty
clones sequenced from various selected organs, the gut

homing peptide YSGKWGK (SEQ ID NO: 9) was present in
22% of the clones; the ovary homing peptides EVRSRLS
(SEQ ID NO: 10) and RVGLVAR (SEQ ID NO: 11) each was
present in 22% of the clones; and the liver homing
5   peptide VKSVCRT (SEQ ID NO: 12) was present in 11% of
the clones (see Table 1). Similarly, the lung homing
peptides CLAKENVVC (SEQ ID NO: 13) and CGFECVRQCPERC
(SEQ ID NO: 1); the skin homing peptide CVALCREACGEGC
(SEQ ID NO: 3); and the retina homing peptide CGEFKVGC
10 (SEQ ID NO: 14) each was independently isolated several
times during *in vivo* panning of the respective organs,
as were other organ homing peptides (see Tables 2
to 11; peptides marked with asterisk). These results
demonstrate that a substantial fraction of the
15 identified organ homing molecules have the same
structure or, in many cases, share conserved motifs.

        Following various *in vivo* panning screens,
hundreds of thousands to millions of phage expressing
homing peptides were recovered from the respective
20 organ or tissue. Generally, the phage collected from a
round of *in vivo* panning were plated on agar, about 250
to 300 clones were selected, grown in 5 ml cultures,
then pooled and readministered for a subsequent round
of *in vivo* panning ("regular method"). However, in
25 some experiments, 1000 clones were selected, grown in 2
ml cultures, then pooled and administered for a
subsequent round of screening; or the entire agar plate
was scraped and all of the phage were cultured together
and administered for a subsequent round of screening.
30 The peptide inserts of various isolated phage were
determined such that, of the millions of phage that
homed, only a small number of sequences were
identified. These results indicate that specific types

of homing molecules can be present in relatively large
proportions in an organ or tissue following *in vivo*
homing, thereby increasing the ease with which the
molecules can be identified.

5   Where an organ or tissue homing molecule is a
nucleic acid molecule, various assay methods can be
used to substantially isolate or identify the molecule.
For example, PCR can be particularly useful for
identifying the presence of the homing molecule
10   because, in principle, PCR can detect the presence of a
single nucleic acid molecule (see, for example, Erlich,
PCR Technology: Principles and Applications for DNA
Amplification (Stockton Press (1989), which is
incorporated herein by reference). PCR also has been
15   used to amplify nucleic acid molecules that bind to a
predetermined target *in vitro* and, when the nucleic
acids were rendered resistant to nucleases and
administered to a subject, they modulated biological
processes such as lymphocyte trafficking *in vivo* (see,
20   for example, Hicke et al., J. Clin. Invest. 98:2688-
2692 (1996), which is incorporated herein by
reference). These findings indicate that nucleic acid
molecules are sufficiently stable when administered
into the circulation of a subject such that *in vivo*
25   panning can be used to identify nucleic acid molecules
that selectively home to an organ or tissue *in vivo*.

The molecules of a library can be tagged,
which can facilitate recovery or identification of the
organ homing molecules. As used herein, the term "tag"
30   means a physical, chemical or biological moiety such as
a plastic or metallic microbead, an oligonucleotide or
a bacteriophage, respectively, that is linked to a

molecule of the library. Methods for tagging a
molecule are well known in the art (Hermanson,
Bioconjugate Techniques, (Academic Press 1996), which
is incorporated herein by reference). The link between
5 a molecule and a tag can be a covalent or a
non-covalent bond and, if desired, the link can be
selectively cleavable from the molecule.

As used herein, the term "shared tag" means a
physical, chemical or biological moiety that is common
10 to each molecule in a library. A shared tag can be
used to identify the presence of a molecule of the
library in a sample or to substantially isolate the
molecules from a sample following in vivo panning. For
example, a library that comprises a population of
15 diverse molecules such as nucleic acids can be linked
to a shared tag. If the shared tag is biotin, for
example, a nucleic acid homing molecule can be
substantially isolated from a selected organ or tissue
by binding, for example, to a streptavidin affinity
20 column. The presence of the organ or tissue homing
nucleic acid molecule also can be detected by binding
with a labeled streptavidin. A peptide such as the
hemagglutinin antigen also can be a shared tag, which,
when linked to each molecule in a library, allows the
25 use of an antibody specific for the hemagglutinin
antigen to substantially isolate homing molecules from
a selected organ or tissue. Furthermore, a molecule or
a support containing a molecule can be linked to a
hapten such as 4-ethoxy-methylene-2-phenyl-2-oxazoline-
30 5-one (phOx), which can be bound by an anti-phOx
antibody linked to a magnetic bead as a means to
recover the homing molecule. Methods for purifying
phOx labeled conjugates are known in the art and the

materials for performing these procedures are
commercially available (Invitrogen, La Jolla CA;
Promega Corp., Madison WI).

A shared tag also can be a nucleic acid
5   sequence that can be used to identify the presence of
molecules of the library in a sample or to
substantially isolate molecules of a library from a
sample. For example, each of the molecules of a
library can be linked to the same selected nucleotide
10 sequence, which constitutes the shared tag. An
affinity column containing a nucleotide sequence that
is complementary to the shared tag then can be used to
isolate the homing molecules from an organ or tissue
sample by hybridizing to the shared tag linked to the
15 molecules. A nucleotide sequence complementary to a
portion of the shared tag also can be used as a PCR
primer such that the presence of molecules containing
the shared tag can be identified in a sample by PCR.

A tag also can be a specific or a unique tag.
20 As used herein, the term "specific tag" means a
physical, chemical or biological tag that is linked to
a molecule in a library and that is unique for the
particular molecule. A specific tag is particularly
useful if it is readily identifiable. A nucleotide
25 sequence that is unique for a particular molecule of a
library is an example of a specific tag, for example,
a unique oligonucleotide tag linked to each peptide of
a library or peptides (see, for example, Brenner and
Lerner, Proc. Natl. Acad. Sci., USA 89:5381-5383
30 (1992), which is incorporated herein by reference).
Upon homing to an organ or tissue, the homing peptide
can be identified by determining the sequence of the

unique oligonucleotide tag using, for example, PCR
(see, for example, Erlich, <u>PCR Technology: Principles</u>
<u>and Applications for DNA Amplification</u> (Stockton Press
1989), which is incorporated herein by reference).
5   Similarly, the nucleic acid sequence encoding a peptide
displayed on a phage is another example of a specific
nucleic acid tag, since sequencing of the nucleic acid
identifies the amino acid sequence of the expressed
peptide (see Example I). Such unique oligonucleotide
10   sequence tags, when linked to other libraries of
molecules, can be used to identify the sequence of the
homing molecule linked thereto.

            A shared tag and specific tag, in
combination, can be particularly useful for isolating
15   and identifying an organ or tissue homing molecule when
the homing molecule is present in minute quantities.
For example, each molecule of a library can be linked
to an oligonucleotide tag which contains two portions;
an internal unique nucleotide sequence tag and shared
20   flanking 5' and 3' nucleotide tags that serve as primer
binding sites for use in PCR. Each molecule,
therefore, contains an oligonucleotide tag having a
unique portion to identify the homing molecule and a
shared portion to provide PCR primer binding sites.
25   Such a tagged molecule, upon homing to a selected organ
or tissue, can be identified by performing PCR using
primers that hybridize to the shared flanking 5' and
3' nucleotide tags, then performing DNA sequencing to
determine the nucleotide sequence of the internal
30   unique sequence tag. The PCR product can be sequenced
directly using one of the PCR primers or the PCR
product can be cloned into a vector and the DNA

sequence determined by routine methods well known in
the art.

Various other combinations of shared and
unique tags can be used. For example, each of the
5   molecules in a library can be linked to a specific
nucleotide sequence tag (see, for example, Brenner and
Lerner, *supra*, 1992), which also contains a shared
3' nucleotide sequence that can be a primer binding
site for use in PCR, and can be further linked to a
10 shared tag such as biotin. Upon homing to an organ or
tissue, the particular homing molecule can be
substantially isolated from an organ or tissue sample
based on the biotin tag. The isolated molecules can
then be identified, for example, by PCR based DNA
15 sequencing of the specific tag using the shared
3' nucleotide sequence of the nucleotide tag as a
primer binding site.

A tag also can serve as a support. As used
herein, the term "support" means a tag having a defined
20 surface to which a molecule can be attached. In
general, a tag useful as a support is a shared tag.
For example, a support can be a biological tag such as
a virus or virus-like particle such as a bacteriophage
("phage"); a bacterium such as *E. coli*; or a eukaryotic
25 cell such as a yeast, insect or mammalian cell; or can
be a physical tag such as a liposome or a microbead,
which can be composed of a plastic, agarose, gelatin or
other biological or artificial material. If desired, a
shared tag useful as a support can have linked thereto
30 a specific tag.

As exemplified herein, a peptide suspected of
being able to home to a selected normal organ or tissue
such as lung, skin, pancreas, retina, prostate, ovary,
lymph node, adrenal gland, liver or gut, or to an organ
5 or tissue containing a tumor, for example, a lung
containing lung tumors or a pancreas containing a   ·
pancreatic tumor, was expressed as the N-terminus of a
fusion protein, wherein the C-terminus consisted of a
phage coat protein (see Example I). Upon expression of
10 the fusion protein, the C-terminal coat protein linked
the fusion protein to the surface of a phage such that
the N-terminal peptide was in a position to interact
with a target molecule in the organ or tissue. Thus, a
molecule having a shared tag was formed by the linking
15 of a peptide to a phage, wherein the phage provided a
biological support, the peptide molecule was linked as
a fusion protein, the phage-encoded portion of the
fusion protein acted as a spacer molecule, and the
nucleic acid encoding the peptide provided a specific
20 tag allowing identification of organ and tissue homing
peptides.

Where a molecule is linked to a support, the
tagged molecule comprises the molecule attached to the
surface of the support, such that the part of the
25 molecule suspected of being able to interact with a
target molecule in a cell in the subject is positioned
so as to be able to participate in the interaction.
For example, where the homing molecule is suspected of
being a ligand for a growth factor receptor, the
30 binding portion of the molecule attached to a support
is positioned so it can interact with the growth factor
receptor on a cell in an organ or tissue. If desired,
an appropriate spacer can be positioned between the

molecule and the support such that the ability of the
potential organ or tissue homing molecule to interact
with the target molecule is not hindered. A spacer
molecule also can contain a reactive group, which
5   provides a convenient and efficient means of linking a
molecule to a support and, if desired, can contain a
tag, which can facilitate recovery or identification of
the molecule (see Hermanson, *supra*, 1996).

            In general, a support should have a diameter
10 less than about 10 µm to about 50 µm in its shortest
dimension, such that the support can pass relatively
unhindered through the capillary beds present in the
subject so as to not occlude circulation. In addition,
a support can be biologically inert, so that it does
15 not perturb the normal expression of cell surface
molecules or normal physiology of the subject. In
addition, a support can be excretable or biodegradable,
particularly where the subject used for *in vivo* panning
is not sacrificed to collect a sample of a selected
20 organ or tissue.

            As used herein, the term "*in vivo* panning,"
when used in reference to the identification of an
organ or tissue homing molecule, means a method of
screening a library by administering the library to a
25 subject and identifying a molecule that selectively
homes to an organ or tissue in the subject (U.S. Patent
No. 5,622,699, *supra*, 1997). The term "administering
to a subject", when used in referring to a library of
molecules or a portion of such a library, is used in
30 its broadest sense to mean that the library is
delivered to a selected organ or tissue in the subject,
which, generally, is a vertebrate, particularly a

mammal such as a human. Libraries of molecules can be
administered by any route or means of administration,
such as intravenously, intramuscularly, orally,
optically, ocularly, intraperitoneally, nasally,
5   vaginally, rectally, into the uterus, into a chamber of
the eye, into the central or peripheral nervous system,
by inhalation, by topical administration, or by
injection into any normal organ or tissue or into a
pathological region such as a tumor or an organ or
10   tissue involved in a pathology, particularly into the
circulatory system of the organ or tissue.

A library can be administered to a subject,
for example, by injecting the library into the
circulation of the subject such that the molecules pass
15   through the selected organ or tissue; after an
appropriate period of time, circulation is terminated,
for example, by perfusion through the heart or by
removing a sample of the organ or tissue (Example I;
U.S. Patent No. 5,622,699, *supra*, 1997; see, also,
20   Pasqualini and Ruoslahti, <u>Nature</u> 380:364-366 (1996),
which is incorporated herein by reference).
Alternatively, a cannula can be inserted into a blood
vessel in the subject, such that the library is
administered by perfusion for an appropriate period of
25   time, after which the library can be removed from the
circulation through the cannula or the subject can be
sacrificed or anesthetized to collect an organ or
tissue sample. A library also can be shunted through
one or a few organs or tissues including a selected
30   organ or tissue, by cannulation of the appropriate
blood vessels in the subject. It is recognized that a
library also can be administered to an isolated
perfused organ or tissue. Such panning in an isolated

perfused organ or tissue can be useful to identify
molecules that bind to the organ or tissue.

The use of *in vivo* panning to identify organ
or tissue homing molecules is exemplified herein by
5   screening a phage peptide display library in mice and
identifying peptides that selectively homed to lung,
pancreas, skin and others, and in rats, for peptides
that homed to retina (Examples I and II). However,
phage libraries that display other protein molecules,
10 including, for example, an antibody or an antigen
binding fragment of an antibody such an Fv, Fd or Fab
fragment; a hormone receptor such as a growth factor
receptor; or a cell adhesion receptor such as an
integrin or a selectin also can be used to practice the
15 invention. Variants of such molecules can be
constructed using well known methods such as random,
site directed or codon based mutagenesis (see Huse,
U.S. Patent No. 5,264,563, issued November 23, 1993,
which is incorporated herein by reference) and, if
20 desired, peptides can be chemically modified, for
example, by introducing a disulfide bridge, following
expression of the phage but prior to administration to
the subject. Thus, many different types of phage
display libraries can be screened by *in vivo* panning.

25 Phage display technology provides a means for
expressing a diverse population of random or
selectively randomized peptides. Various methods of
phage display and methods for producing diverse
populations of peptides are well known in the art. For
30 example, Ladner et al. (U.S. Patent No. 5,223,409,
issued June 29, 1993, which is incorporated herein by
reference) describe methods for preparing diverse

populations of binding domains on the surface of a
phage. In particular, Ladner et al. describe phage
vectors useful for producing a phage display library,
as well as methods for selecting potential binding
5 domains and producing randomly or selectively mutated
binding domains.

Similarly, Smith and Scott (<u>Meth. Enzymol.</u>
217:228-257 (1993); see, also, Scott and Smith, <u>Science</u>
249: 386-390 (1990), each of which is incorporated
10 herein by reference) describe methods of producing
phage peptide display libraries, including vectors and
methods of diversifying the population of peptides that
are expressed (see, also, Huse, WO 91/07141 and
WO 91/07149, each of which is incorporated herein by
15 reference; see, also, Example I). Phage display
technology can be particularly powerful when used, for
example, with a codon based mutagenesis method, which
can be used to produce random peptides or randomly or
desirably biased peptides (Huse, U.S. Patent No.
20 5,264,563, *supra*, 1993). These or other well known
methods can be used to produce a phage display library,
which can be subjected to the *in vivo* panning method of
the invention in order to identify a peptide that homes
to a selected organ or tissue.

25 In addition to screening a phage display
library, *in vivo* panning can be used to screen various
other types of libraries. For example, nucleic acid
molecules that bind to a cell surface receptor have
been described (see O'Connell et al., <u>Proc. Natl. Acad.</u>
30 <u>Sci., USA</u> 93:5883-5887 (1996); Tuerk and Gold, <u>Science</u>
249:505-510 (1990); Gold et al., *supra* (1995), each of
which is incorporated herein by reference). These

in vitro results indicate that a library of nucleic acid molecules also can be examined by *in vivo* panning to identify nucleic acid molecules that home to a selected organ or tissue. Additional libraries

5   suitable for screening include, for example, oligosaccharide libraries (York et al., Carb. Res. 285:99-128, (1996); Liang et al., Science 274:1520-1522, (1996); and Ding et al., Adv. Expt. Med. Biol. 376:261-269, (1995), each of which is

10 incorporated by reference); lipoprotein libraries (de Kruif et al., FEBS Lett. 399:232-236, (1996), which is incorporated herein by reference); glycoprotein or glycolipid libraries (Karaoglu et al., J. Cell Biol. 130:567-577 (1995), which is incorporated herein by

15 reference); or chemical libraries containing, for example, drugs or other pharmaceutical agents (Gordon et al., J. Med. Chem. 37:1385-1401 (1994); Ecker and Crook, Bio/Technology 13:351-360 (1995); each of which is incorporated by reference). Such

20 libraries, if desired, can be tagged, which can facilitate recovery of the molecule from an organ or tissue or its identification as previously described.

In vivo panning provides a method for directly identifying molecules that can selectively

25 home to an organ or tissue. As used herein, the term "home" or "selectively home" means that a particular molecule binds relatively specifically to a target molecule present in the organ or tissue, particularly in the vasculature present in the organ or tissue,

30 following administration to a subject. In general, selective homing is characterized, in part, by detecting at least a two-fold (2x) greater selective

binding of the molecule to an organ or tissue as
compared to a control organ or tissue.

Selective homing of a molecule to a selected
organ or tissue can be due to selective recognition by
5 the molecule of a particular cell target molecule such
as a cell surface protein present on a cell in the
organ or tissue. Selectivity of homing is dependent on
the particular target molecule being expressed on only
one or a few different cell types, such that the
10 molecule homes to only one or a few organs or tissues.
In this regard, most different cell types, particularly
cell types that are unique to an organ or tissue, can
express unique target molecules. Thus, in organs such
as liver, spleen or lymph node, where blood circulates
15 through sinusoids formed by the cells specific for the
organ, *in vivo* panning can be useful for identifying
molecules that home to the particular organ or tissue.

It should be recognized that, in some
instances, a molecule can localize nonspecifically to
20 an organ or tissue. For example, *in vivo* panning of a
phage display library can result in high background in
organs such as liver and spleen, which contain a marked
component of the reticuloendothelial system (RES).
Thus, nonspecific binding of molecules due to uptake by
25 the RES of such an organ or tissue can make identifying
an organ or tissue homing molecule more difficult.
However, as disclosed herein, potential nonspecific
binding can be circumvented, for example, by perfusion
through the heart prior to collecting the selected
30 organ or tissue (Example I).

In addition, selective homing readily can be distinguished from nonspecific binding by detecting differences in the abilities of different individual phage to home to an organ or tissue. For example,
5   selective homing can be identified by combining a putative homing molecule such as a peptide expressed on a phage with an excess of non-infective phage or with about a five-fold excess of phage expressing unselected peptides, injecting the mixture into a subject and
10 collecting a sample of the organ or tissue. In the latter case, for example, provided the portion of injected phage in which an organ or tissue homing peptide is sufficiently low so as to be nonsaturating for the target molecule, a determination that greater
15 than about 20% of the phage in the organ or tissue contain the putative homing molecule is demonstrative evidence that the peptide expressed by the phage is a selective organ or tissue homing molecule. In addition, nonspecific localization can be distinguished
20 from selective homing by performing competition experiments using, for example, phage expressing a putative organ or tissue homing peptide in combination with an excess amount of the "free" peptide (see Example II).

25   Various methods can be used to prevent nonspecific localization of a molecule to organs or tissues, such as those containing a component of the RES. For example, as disclosed herein, perfusion of a solution through the heart shortly after initiating
30 phage circulation decreased the background binding and allowed identification of peptides that selectively home to lung and liver, both of which contain a component of the RES (see Example II). Furthermore,

coadministration of nonreplicating control phage with a
phage display library reduced nonspecific phage
trapping in organs such as liver and spleen, which also
contain a component of the RES. This approach allowed
5   identification of molecules that selectively home to
liver (Example II). Thus, a library of molecules
attached to a support can be coadministered with an
excess of the support to a subject to inhibit
nonspecific binding in an organ or tissue.

10   Nonspecific uptake by a component of the RES
also can be prevented by administering a blocking agent
that inhibits uptake by the RES. For example,
polystyrene latex particles or dextran sulfate can be
administered to the subject prior to the administration
15   of the library (see Kalin et al., Nucl. Med. Biol.
20:171-174 (1993); Illum et al., J. Pharm. Sci.
75:16-22 (1986); Takeya et al., J. Gen. Microbiol.
100:373-379 (1977), each of which is incorporated
herein by reference). Such pre-administration of
20   dextran sulfate 500 or polystyrene microspheres has
been used to block nonspecific uptake of a test
substance by Kupffer cells, which are the RES component
of the liver (Illum et al., *supra*, 1986). Similarly,
nonspecific uptake of agents by the RES has been
25   blocked using carbon particles or silica (Takeya et
al., *supra*, 1977) or a gelatine colloid (Kalin et al.,
supra, 1993). Thus, many methods useful for inhibiting
nonspecific uptake by the RES are known in the art and
routinely used.

30   Methods of decreasing nonspecific phage
trapping include using phage that display a low
background binding to a particular organ or tissue.

For example, Merrill et al. (_Proc. Natl. Acad. Sci.,_
USA 93:3188-3192 (1996), which is incorporated herein
by reference) selected lambda-type phage that are not
taken up by the RES and, as a result, remain in the
5   circulation for a prolonged period of time. A
comparable filamentous phage variant, for example, can
be selected using similar methods.

Selective homing can be demonstrated by
determining if a homing molecule for a selected organ
10   or tissue is relatively specific. For example, the
amount of homing molecule in a selected organ or tissue
can be compared to a control or different organ or
tissue. Selective homing also can be demonstrated by
showing that molecules that home to an organ or tissue,
15   as identified by one round of _in vivo_ panning, are
enriched for in a subsequent round of _in vivo_ panning.
For example, phage expressing the peptides
CGFECVRQCPERC (SEQ ID NO: 1) and CGFELETC (SEQ ID
NO: 2) were enriched for in the second and third rounds
20   of _in vivo_ panning from lung and exhibited a 35-fold
and 9-fold enrichment, respectively, as compared to
unselected phage (see Example II.B). Furthermore, no
selective homing to kidney or brain was detected.

As used herein, the term "selected organ or
25   tissue" is used in its broadest sense to mean a normal
organ or tissue or an organ or tissue having a
pathology, for example, lung containing lung tumors, to
which a molecule can selectively home. Thus, the term
"organ or tissue" is used broadly to mean any tissue or
30   organ including a normal or pathological cell type such
as a cancer cell, in which case the selected organ or
tissue can be a primary tumor or a metastatic lesion.

In general, a selected organ or tissue
contains a cell, which can be a cell of the
vasculature, that expresses a particular target
molecule such as a cell surface protein to which a
5   homing molecule can bind. By performing at least two
rounds of *in vivo* panning, the selectivity of homing of
the molecule for the selected organ or tissue can be
determined. As discussed below, however, in some cases
a homing molecule can home to more than one selected
10 organ or tissue, in which case the molecule is
considered to be able to selectively home to a family
of selected organs or tissues. Generally, however,
molecules that home to more than one or a few different
organs or tissue are not particularly useful since an
15 advantage of the homing molecules of the invention is
that they allow targeting of a particular organ or
tissue.

The term "control organ or tissue" is used to
mean an organ or tissue other than the selected organ
20 or tissue. A control organ or tissue is characterized
by the inability of the organ or tissue homing molecule
to home to the control organ or tissue and, therefore,
is useful for identifying selective binding of a
molecule to a selected organ or tissue (Example II).
25 Where an organ or tissue homing molecule is identified
based on its ability to home to a pathologic lesion in
an organ or tissue, the control organ or tissue can be
a corresponding portion of the selected organ or tissue
that does not exhibit the pathologic lesion.

30   A control organ or tissue can be collected,
for example, to identify nonspecific binding of the
molecule or to determine the selectivity of homing of

the molecule. In addition, nonspecific binding can be
identified by administering, for example, a control
molecule, which is known not to home to an organ or
tissue but is chemically similar to a putative homing

5 molecule. Alternatively, where the administered
molecules are linked to a support, administration of
the support, alone, can be used to identify nonspecific
binding. For example, a phage that does not contain a
peptide fusion protein can be administered to a subject

10 and the selected organ or tissue can be examined to
determine the level of nonspecific binding of the phage
support.

    The steps of administering the library to the
subject, collecting a selected organ or tissue and

15 identifying the molecules that home to the organ or
tissue, comprise a single round of *in vivo* panning.
Although not required, one or more additional rounds of
in vivo panning generally are performed. Where an
additional round of *in vivo* panning is performed, the

20 molecules recovered from the selected organ or tissue
in the previous round are administered to a subject,
which can be the same subject used in the previous
round, where only a part of the organ or tissue was
collected.

25   By performing a second round of *in vivo*
panning, the relative binding selectivity of the
molecules recovered from the first round can be
determined by administering the identified molecules to
a subject, collecting the selected organ or tissue, and

30 determining whether more phage displaying a particular
molecule are recovered from the organ or tissue
following the second round of screening as compared to

those recovered following the first round. Although not required, a control organ or tissue also can be collected and the molecules recovered from the selected organ or tissue can be compared with those recovered 5 from the control organ or tissue. Ideally, few if any molecules are recovered from a control organ or tissue following a second or subsequent round of *in vivo* panning. Generally, however, a proportion of the molecules also will be present in a control organ or 10 tissue. In this case, the ratio of molecules in the selected organ or tissue as compared to the control organ or tissue (selected:control) can be determined. Additional rounds of *in vivo* panning can be used to determine whether a particular molecule homes only to 15 the selected organ or tissue or can recognize a target expressed in one or more other organs or tissues that is identical or is sufficiently similar to the target in the originally selected organ or tissue.

In general, a library of molecules, which 20 contains a diverse population of random or selectively randomized molecules of interest, is prepared, then administered to a subject. Some time after administration, the selected organ or tissue is collected and the molecules present in the selected 25 organ or tissue are identified (see Example I). If desired, one or more control organs or tissues or a part of a control organ or tissue are sampled as well. For example, mice injected with a phage peptide display library, after about 1 to 5 minutes, were anesthetized, 30 then snap frozen or perfused through the heart to terminate circulation of the phage. Lung, pancreas or other organs or tissues and one or more control organs were collected and the phage present in the selected

and control organs were collected. The peptides that
selectively homed to the respective organs or tissues
were identified (Example II and Tables 1 to 11).

As exemplified herein, experimental animals
5   were sacrificed to collect the selected or control
organ or tissue. It should be recognized, however,
that only a part of an organ or tissue need be
collected to recover a molecule that homes to that
organ or tissue. Similarly, only part of an organ or
10 tissue need be collected as a control. Thus, for
example, following administration of a library of
molecules to a subject, a part of the selected organ or
tissue can be collected by biopsy, the homing molecules
can be collected and, if desired, amplified and
15 readministered to the same subject for a second round
of *in vivo* panning. Where the molecule that is to be
administered a second time to the same subject is
tagged or linked, for example, to a support, the tag or
support should be biologically inert and biodegradable
20 or excretable, so as not to interfere with subsequent
rounds of screening.

In vitro screening of phage libraries
previously was used to identify peptides that bind to
antibodies or to cell surface receptors (Smith and
25 Scott, *supra*, 1993). For example, *in vitro* screening
of phage peptide display libraries identified novel
peptides that specifically bound to integrin adhesion
receptors (Koivunen et al., J. Cell Biol. 124:373-380
(1994a), which is incorporated herein by reference) and
30 to the human urokinase receptor (Goodson et al., Proc.
Natl. Acad. Sci., USA 91:7129-7133 (1994), which is
incorporated herein by reference). Similarly, *in vitro*

screening of nucleic acid molecules identified
molecules that specifically bind to antibodies, cell
surface receptors or organic molecules (Gold et al.,
supra, 1993, 1995, 1997). For example, RNA molecules
5   that specifically bind to HIV-1 reverse transcriptase
were identified using purified HIV-1 reverse
transcriptase as the target molecule (Green et al.,
J. Mol. Biol., 247:60-68 (1995), which is incorporated
herein by reference). These *in vitro* methods were
10 performed using defined, well-characterized target
molecules in an artificial system. However, such
in vitro studies provide no insight as to whether a
molecule that binds *in vitro* also can bind to the
target *in vivo*. For example, endothelial cells grown
15 in culture tend to lose their tissue-specific
differences (Pauli and Lee, Lab. Invest. 58:379-387
(1988), which is incorporated herein by reference).
Thus, a molecule that binds to a target on a cell *in
vitro* may not bind *in vivo* because the target may not
20 be present on the cell. Furthermore, such *in vitro*
methods are limited in that they require prior
knowledge of the target molecule and yield little if
any information regarding *in vivo* utility. For
example, Goodson et al. (*supra*, 1994) utilized cultured
25 cells to express a recombinant urokinase receptor to
obtain binding peptides. However, the urokinase
receptor is expressed in cells of many different organs
and tissues and, therefore, a molecule that binds to it
can interact with many organs or tissues and would not
30 be considered an organ or tissue homing molecule within
the present invention.

In contrast to *in vitro* panning methods, *in
vivo* panning requires no prior knowledge or the

availability of a known target molecule to identify a
molecule that binds to a target molecule that is
expressed *in vivo*. Also, since "nontargeted" organs or
tissues are present during the screening, the
5 probability of isolating organ or tissue homing
molecules that lack selectivity of homing is greatly
reduced. Furthermore, in obtaining organ or tissue
homing molecules by *in vivo* panning, any molecules that
may be particularly susceptible to degradation in the
10 circulation *in vivo* due, for example, to a metabolic
activity, will be selected against and will not be
recovered. Thus, *in vivo* panning provides significant
advantages over previous methods by identifying
molecules that selectively home *in vivo* and, if
15 desired, the target molecule present on a selected
organ or tissue.

The identification of the organ homing
molecules that selectively home to various normal
tissues and to pathologic lesions in a particular organ
20 or tissue, as exemplified herein, indicates that
particular endothelial cell target molecules expressed
the selected organ or tissue reflects the physiologic
or pathologic state of the organ or tissue. Such organ
homing molecules that selectively home to an organ or
25 tissue based on a particular physiologic or pathologic
condition occurring in the organ or tissue can be
identified using the *in vivo* panning method and the
selectivity of the homing molecules for the pathologic
or physiologic condition of the organ or tissue can be
30 confirmed by immunohistological analysis (Example III).
For example, molecules that home to pancreas afflicted
with pancreatitis can be identified by *in vivo* panning
of a subject having pancreatitis and selectively of the

homing molecule can be confirmed by using immunohistochemistry to compare homing of the molecule in normal pancreas with homing in a pancreas afflicted with pancreatitis.

5   Homing molecules selective for a normal organ or tissue or an organ or tissue exhibiting a pathological state can be useful for detecting the presence or absence of the pathology. For example, following administration of a prostate homing molecule

10   conjugated to an imaging moiety to a subject, the prostate can be visualized. If the image is abnormal, for example, if the size of the prostate is other than that expected for a size and age matched subject, the imaging result can indicate an abnormal physiologic

15   condition or pathologic condition afflicting the prostate. For example, a conjugate comprising an imaging agent and a prostate homing molecule that homes to normal, but not to abnormal prostate, can be administered to a subject. The identification, for

20   example, of a region of the prostate that does not bind the homing molecule can indicate the occurrence of abnormal blood flow in the prostate and can be diagnostic of a pathologic condition such as the presence of a prostate tumor. A conjugate comprising a

25   molecule that homes to prostate tumor tissue, but not to normal prostate, can be used to image a prostate tumor directly.

      A homing molecule selective for an organ or tissue can be used to deliver a therapeutic agent to

30   the organ or tissue. Such selective targeting of the agent can increase the effective amount of the agent delivered to the target organ or tissue, while reducing

the likelihood the agent will have an adverse effect on
other organs or tissues. For example, a lung homing
molecule can be used to deliver, to the lung of a
cystic fibrosis patient, a gene encoding the cystic
5   fibrosis transmembrane receptor (CFTR), which is
defective in cystic fibrosis. Thus, the organ homing
molecules of the invention are particularly useful for
in vivo gene therapy, since they provide a means to
direct a gene to a desired target organ, thereby
10 increasing the likelihood that the target cells will
receive the gene and decreasing the likelihood that
normal, nontarget, cells will be adversely affected. A
lung homing molecule also can be used to direct a
therapeutic agent to the lung, thus sparing nontarget
15 organs or tissues from the toxic effects of the agent.
For example, in alveolar bacterial pneumonia, a lung
homing molecule can be useful for directing an
antibiotic to the afflicted region of the lung, thus
increasing the effective amount of the drug at the
20 desired site.

Due to the conserved nature of cellular
receptors and of ligands that bind a particular
receptor, the skilled artisan would recognize that an
25 organ or tissue homing molecule identified using
in vivo panning in a mouse or rat also can bind to the
corresponding target molecule in the selected organ or
tissue of a human or other mammalian species. Such a
homing molecule identified using an experimental animal
30 readily can be examined for the ability to bind to the
corresponding organ or tissue in a human subject by
demonstrating, for example, that the molecule also can
bind selectively *in vitro* to a sample of the selected
organ or tissue obtained from a human subject.

Alternatively, primary cells or established cell lines derived from a human organ or tissue can be used to test for the *in vitro* binding of the homing molecule. Similarly, primary cells or established cell lines that
5 reflect a particular human organ or tissue pathology can be used to test the binding of homing molecules selective for the pathology. Animal models such as primate models of human pathologies are known and also can be used to test for the homing of the molecules
10 using *in vivo* panning. Thus, routine methods can be used to confirm that an organ or tissue homing molecule identified using *in vivo* panning in an experimental animal also can bind an organ or tissue-specific target molecule in a human subject. Furthermore, *in vitro*
15 contacting of a homing molecule with a sample suspected of containing a selected organ, tissue or pathology can identify the presence of the selected organ, tissue or pathology in the sample. Having identified the target molecule by *in vivo* panning, the artisan would know
20 that it is the true target for an organ homing molecule and, therefore, would know that the target molecule could be used *in vitro* to identify additional organ homing molecules that likely would be specific for the target molecule *in vivo*. Such potential organ homing
25 molecules then could be examined by *in vivo* panning to confirm organ homing ability.

In *vivo* panning was used to identify peptides expressed by phage that selectively homed to lung, skin, pancreas, retina, prostate, ovary, lymph node,
30 adrenal gland, liver or gut, and to lung containing lung tumors or pancreas containing a pancreatic tumor (Examples II and IV; see, also, Tables 2 to 11). Due to the large size of the phage (300 nm) and the short

time the phage were allowed to circulate, it is
unlikely that a substantial number of phage would have
exited the circulatory system. Indeed,
immunohistochemical studies of various organ and tissue
5   homing molecules demonstrated that the molecules
primarily home to and bind endothelial cell surface
markers of the vasculature. Thus, the invention
provides molecules such as peptides that selectively
home to the vasculature of a selected organ or tissue.

10   Phage peptide display libraries were
constructed essentially as described by Smith and Scott
(*supra*, 1993; see, also, Koivunen et al., Biotechnology
13:265-270 (1995); Koivunen et al., Meth. Enzymol.
245:346-369 (1994b), each of which is incorporated
15   herein by reference). In some libraries, at least one
codon encoding cysteine also was included in each
oligonucleotide so that cyclic peptides could be formed
through disulfide linkages (Example I). Upon
performing *in vivo* panning, peptides that selectively
20   home to lung, pancreas, skin, retina, prostate, ovary,
lymph node, adrenal gland, liver or gut or to lung
containing lung tumors or to pancreas containing a
pancreatic tumor were obtained. Thus, the invention
provides various organ homing molecules that
25   selectively home to particular organs or tissue.

           Remarkably, some organ homing peptides
independently were recovered up to four or more times
during a round of the *in vivo* panning procedure (see,
for example, Table 1). In addition, various peptides
30   that homed to particular organs or tissues shared
conserved amino acid sequence motifs. For example,
some lung homing peptides shared a GFE motif; some

TABLE 1

SUMMARY OF IN VIVO TARGETING OF VARIOUS ORGANS

ORGAN/MOTIF (SEQ ID NO:)	% OF MOTIF AMONG ALL CLONES	LUNG/BRAIN RATIO
GUT		
YSGKWGK (9)	22	30
GISALVLS (19)	11	nd
SRRQPLS (153)	11	2
MSPQLAT (159)	11	nd
MRRDEQR (172)		
QVRRVPE (155)		
VRRGSPQ (164)		
GGRGSWE (167)		
FRVRGSP (169)		
RVRGPER (165)		
LIVER		
VKSVCRT (12)	11	nd
WRQNMPL (418)	6	nd
SRRFVGG (406)	6	nd
ALERRSL (408)		
ARRGWTL (405)		
PROSTATE		
SMSIARL (21)	6	34
VSFLEYR (22)	6	17
RGRWLAL (279)	6	nd
ADRENAL GLAND		
LMLPRAD (27)	11	50
LPRYLLS (28)		
R(Y/F)LLAGG (404)		
RYPLAGG (389)		

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ORGAN/MOTIF (SEQ ID NO:)	% OF MOTIF AMONG ALL CLONES	LUNG/BRAIN RATIO
OVARY		
EVRSRLS (10)	22	3
FFAAVRS (295)		
VRARLMS (301)		
RVGLVAR (11)	22	5
RVRLVNL (294)		
PANCREAS		
SWCEPGWCR (4)		20
SKIN	9	
CVALCREACGEGC (3)	6	7
CSSGCSKNCLEMC (181)		2
LUNG		
CTLRDRNC (15)	10	8
CGKRYRNC (20)	5	5
CLRPYLNC (45)	10	6
CGFELETC (2)	5	9
CIGEVEVC (16)	5	6
CKWSRLHSC (65)	11	3
CWRGDRKIC (56)	8	2
CERVVGSSC (59)	9	4
CLAKENVVC (13)	13	2
CTVNEAYKTRMC (75)	22	3
CRLRSYGTLSLC (76)	5	0.4
CRPWHNQAHTEC (82)	14	5
CGFECVRQCPERC (1)	40	60

retina homing peptides shared a RDV motif; and some
adrenal gland homing peptides shared a LPR motif (see
Tables 2, 6 and 11, respectively). Since it is known,
for example, that the tripeptide RGD motif is
5   sufficient for integrin binding (Ruoslahti, Ann. Rev.
Cell Devel. Biol. 12:697 (1996); Koivunen et al.,
supra, 1995; WO 95/14714), the results disclosed herein
indicate that many ligand/receptor interactions can
derive their specificity from recognition motifs as
10 small as tripeptides.

None of the sequences of the disclosed organ
homing peptides exhibited significant similarity with
known ligands for endothelial cell receptors. While
many of the organ homing peptides may be contained
15 within larger peptides or proteins, it is not known
whether they are able to impart a homing function onto
the larger molecule. . Based on the previous finding
that RGD mediates integrin binding when present within
larger peptides and proteins, one skilled in the art
20 would recognize, however, that such homing peptides and
motifs could impart a homing function when located
within a larger peptide or protein. However, such
naturally occurring endogenous peptides and proteins
are not considered to be organ or tissue homing
25 molecules within the invention.

The organ or tissue homing peptide molecules
exemplified herein range in size from about 7 to 13
amino acids in length. However, based, for example, on
the ability of the RGD integrin binding motif to
30 mediate integrin binding by itself or when present in a
large protein, it will be recognized that the organ
homing molecules of the invention also can be expected

to maintain their homing capability in the context of a
significantly longer polypeptide sequence. Thus, an
organ homing peptide of the invention can be at least
three amino acids, generally at least six amino acids
5   or seven amino acids or more, and can be significantly
larger, for example, about 20 to 50 amino acids or
100 amino acids or more.

      The invention provides lung homing peptides
such as CGFECVRQCPERC (SEQ ID NO: 1) and CGFELETC (SEQ
10   ID NO: 2), which share a GFE motif; CTLRDRNC (SEQ ID
NO: 15); and CIGEVEVC (SEQ ID NO: 16; see Table 1),
which contains an EVE motif that is similar to the ELE
motif present in CGFELETC (SEQ ID NO: 2). The
exemplified lung homing peptides were identified by
15   injection of a $CX_3CX_3CX_3C$, (SEQ ID NO: 25), CX_7C (SEQ ID
NO: 24) or CX_6C (SEQ ID NO: 26) cyclic library into
mice (Example II). The lung homing peptides
CGFECVRQCPERC (SEQ ID NO: 1) and CGFELETC (SEQ ID
NO: 2) exhibited a 60-fold and 9-fold enrichment,
20   respectively, as compared to unselected phage, with few
phage detected in kidney or brain (Example II; see,
also, Figures 1 and 2 and Table 1). In addition, the
lung homing peptides CTLRDRNC (SEQ ID NO: 15) and
CIGEVEVC (SEQ ID NO: 16) exhibited a 8-fold and 6-fold
25   enrichment, respectively, over unselected phage
(Table 1). Coinjection of a glutathione-S-transferase-
(GST-)CGFECVRQCPERC (SEQ ID NO: 1) fusion peptide with
phage expressing the cognate CGFECVRQCPERC (SEQ ID
NO: 1) peptide inhibited homing by 70%, and coinjection
30   of GST-CGFELETC (SEQ ID NO: 2) with phage expressing
(SEQ ID NO: 2) inhibited lung homing by 30% (Figure 3).
Immunohistochemical staining of lung following
administration of phage displaying a lung homing

peptide to mice revealed staining within the alveolar
capillaries. No apparent preference for homing of the
phage to any particular region of the lung was
observed; however, no staining was observed in
5   bronchioles luminal walls or some larger blood vessels
(Example III), or in many other tissues analyzed.
These results indicate that *in vivo* panning can be used
to identify and analyze endothelial cell specificities
within lung, thus providing a means to differentially
10 target lung.

The invention also provides skin homing
peptides such as CVALCREACGEGC (SEQ ID NO: 3; Table 5),
which were identified by injection of a $CX_3CX_3CX_3C$ (SEQ
ID NO: 25) cyclic library into mice (Example II). The
15 skin homing peptide sequence CVALCREACGEGC (SEQ ID
NO: 3) exhibited a 7-fold selectivity for skin over
unselected phage and over background in brain and
kidney (Figure 2; see, also, Table 1). Coinjection of
GST-CVALCREACGEGC (SEQ ID NO: 3) with phage expressing
20 CVALCREACGEGC (SEQ ID NO: 3) inhibited homing to skin
by 55%, whereas coinjection with GST, alone, had no
effect on homing (see Figure 3B). Immunohistochemical
staining of skin following administration of phage
displaying a skin homing peptide revealed that staining
25 was localized to the hypodermis; no staining was
observed in the dermis (Example III).

The invention further provides pancreas
homing peptides such as SWCEPGWCR (SEQ ID NO: 4;
Table 3). The exemplified pancreas homing molecules
30 were identified by injection of a CX_7C (SEQ ID NO: 24)
or X_2CX_4CX (SEQ ID NO: 23) cyclic library into mice
(Example II). The pancreas homing peptide SWCEPGWCR

(SEQ ID NO: 4) exhibited a 20-fold selectivity for pancreas over unselected phage and over brain (Table 1; Figure 2). However, coinjection of GST-SWCEPGWCR (SEQ ID NO: 4) did not inhibit SWCEPGWCR (SEQ ID NO: 4)

5 pancreas homing, presumably due to a conformational effect of GST on the pancreas homing peptide. Immunohistochemical staining of pancreas following administration of phage displaying a pancreas homing peptide revealed that staining was localized to the

10 capillaries as well as larger blood vessels of the exocrine pancreas; no significant staining was observed in the endocrine vasculature (Example III). This result demonstrates that histologically and physiologically distinguishable regions within a

15 particular organ can express unique target molecules, which provide a target for an organ homing molecule of the invention. Accordingly, the organ homing molecules of the invention provide a means to differentially targeted specific regions of a selected organ or

20 tissue.

Retina homing peptides such as CSCFRDVCC (SEQ ID NO: 5) and CRDVVSVIC (SEQ ID NO: 6) also are provided (see Table 6). The exemplified retina homing molecules were identified by injection of a CX_7C (SEQ

25 ID NO: 24) cyclic library into rats (Example II). The retina homing peptides CSCFRDVCC (SEQ ID NO: 5) and CRDVVSVIC (SEQ ID NO: 6), when injected individually with a control fdAMPLAY88 phage, exhibited a 3-fold and 2-fold enrichment, respectively, in retina

30 (Example II). However, immunohistochemical staining revealed an absence of retina staining, presumably due to a relatively modest accumulation of the retina homing phage in the target tissue.

The invention also provides prostate homing peptides such as SMSIARL (SEQ ID NO: 21) and VSFLEYR (SEQ ID NO: 22), which were identified by injection of an X7 (SEQ ID NO: 29) library into mice (Table 7). The

5 peptides were isolated by the regular method. The prostate homing peptides SMSIARL (SEQ ID NO: 21) and VSFLEYR (SEQ ID NO: 22) exhibited a 34-fold and 17-fold enrichment, respectively, in homing to prostate as compared to brain (Table 1).

10 Also provided are ovary homing peptides such as RVGLVAR (SEQ ID NO: 11) and EVRSRLS (SEQ ID NO: 10), which were identified by injection of an X7 (SEQ ID NO: 29) library into mice (Table 8). The peptides were isolated by the regular method. The ovary homing

15 peptides RVGLVAR (SEQ ID NO: 11) and EVRSRLS (SEQ ID NO: 10) each comprised 22% of 40 clones sequenced and exhibited a 5-fold and a 3-fold enrichment, respectively, in ovary as compared to brain (Table 1).

The invention also provides adrenal gland

20 homing peptides such as LMLPRAD (SEQ ID NO: 27) and LPRYLLS (SEQ ID NO: 28), which share a LPR motif (see Table 11), or the peptides R(Y/F)LLAGG (SEQ ID NO: 404) and RYPLAGG (SEQ ID NO: 389), which share the motif LAGG (SEQ ID NO: 430; see Table 10). The exemplified

25 adrenal gland homing peptides were identified by injection of an X7 (SEQ ID NO: 29) library into mice. The peptides were isolated by the regular method. The adrenal gland homing peptide LMLPRAD (SEQ ID NO: 27) exhibited a 50-fold enrichment in adrenal gland as

30 compared to brain (Table 1).

Also provided are liver homing peptides.
Such peptides were identified by injection of an X_7
(SEQ ID NO: 29) library into mice. The peptides were
isolated by the regular method (see Example II,
5   Table 1, and Table 11, below).

In addition, lymph node homing peptides, such
as AGCSVTVCG (SEQ ID NO: 315) are provided (Table 9,
below). Such peptides were identified by injection of
an X_2CX_4CX (SEQ ID NO: 23) library into mice. The
10   peptides were isolated by the regular method.

The invention also provides gut homing
peptides such as YSGKWGK (SEQ ID NO: 9) and YSGKWGW
(SEQ ID NO: 156), which were identified by injection of
an X_7 (SEQ ID NO: 29) library into mice (Tables 1 and
15   4) and differ only in the last amino acid position.
The peptides were isolated by the regular method. The
gut homing peptide YSGKWGK (SEQ ID NO: 9) was present
in 22% of 40 clones sequenced and was enriched 30-fold
in gut as compared to brain (Table 1). In addition,
20   gut homing peptides such as QVRRVPE (SEQ ID NO: 155)
and VRRGSPQ (SEQ ID NO: 164), which share a VRR motif,
were identified, as were the peptides VRRGSPQ (SEQ ID
NO: 164), GGRGSWE (SEQ ID NO: 167) and FRVRGSP (SEQ ID
NO: 169), which share an RGS motif.

25   The organ homing molecules of the invention
are particularly useful as conjugates, which comprise
the organ homing molecule linked to a moiety. Thus, a
lung, skin, pancreas, retina, prostate, ovary, lymph
node, adrenal gland, liver or gut homing molecule of
30   the invention can be linked to a moiety, such

conjugates being useful for directing the moiety to the particular selected organ.

As used herein, the term "moiety" is used broadly to mean a physical, chemical, or biological
5   material that is linked to an organ or tissue homing molecule. Generally, a moiety linked to an organ homing molecule imparts a biologically useful function to the homing molecule. A moiety can consist of any natural or nonnatural material for example, peptide or
10 polypeptide sequences, organic or inorganic molecules or compositions, nucleic acid molecules, carbohydrates, lipids or combinations thereof.

A moiety can be a physical, chemical or biological material such as a virus, viral gene therapy
15 vector, cell, liposome, microcapsule, micropump or other chambered microdevice, which can be used, for example, as a drug delivery system. Generally, such microdevices should be biologically inert and, if desired, biodegradable or excretable. Various
20 moieties, including microcapsules, which can contain an agent, and methods for linking a moiety or chambered microdevice to an organic molecule of the invention are well known in the art and commercially available (see, for example, "Remington's Pharmaceutical Sciences" 18th
25 ed. (Mack Publishing Co. 1990), chapters 89-91; Harlow and Lane, Antibodies: A laboratory manual (Cold Spring Harbor Laboratory Press 1988), each of which is incorporated herein by reference; see, also, Hermanson, supra, 1996). Additional examples of moieties are
30 known to those skilled in the art and are intended to be included within the meaning of the term so long as

they possess a biologically useful function when linked
to the homing molecules of the invention.

Linking of a moiety to an organ homing
molecule for the purpose of directing the moiety to the
5 selected organ or tissue was demonstrated by the
linking of a brain homing peptide to a red blood cell
(RBC), wherein the peptide directed homing of the RBC
to the brain (U.S. Patent No. 5,622,699, *supra*, 1997).
These results indicate that an organ or tissue homing
10 molecule of the invention can be linked to another
moiety in order to direct the moiety to a selected
organ or tissue. For example, a liver homing molecule
or a lung homing molecule can be linked to a nucleic
acid encoding the CFTR gene and upon administration to
15 a subject, expression of CFTR is targeted to the liver
or to the lung, respectively. Similarly, a lung homing
molecule can be linked to a protease inhibitor such
that, upon administration of the conjugate comprising
the lung homing molecule and the protease inhibitor to
20 a subject, the protease inhibitor is targeted to the
lung. Such a conjugate can be useful, for example, for
treating a subject suffering from emphysema, which is
characterized by excessive protease production in the
lungs and autodigestion of the organ.

25 An organ and tissue homing molecule of the
invention can be useful for directing to a selected
organ or tissue a therapeutic agent, diagnostic agent
or imaging agent, a tag or insoluble support, a
liposome or a microcapsule comprising, for example, a
30 permeable or semipermeable membrane, wherein an agent
such as a drug to be delivered to a selected organ or
tissue is contained within the liposome or

microcapsule. These and other moieties known in the
art can be used in a conjugate of the invention, and in
a method of the invention, as disclosed herein.

   In one embodiment, a moiety can be a
5 detectable agent such as a radionuclide or an imaging
agent, which allows detection or visualization of the
selected organ or tissue. Thus, the invention provides
a conjugate comprising a lung, skin, pancreas, retina,
prostate, ovary, lymph node, adrenal gland, liver or
10 gut homing molecule, linked to a detectable agent. The
type of detectable agent selected will depend upon the
application. For example, for an *in vivo* diagnostic
imaging study of the lung in a subject, a lung homing
molecule can be linked to an agent that, upon
15 administration to the subject, is detectable external
to the subject. For detection of such internal organs
or tissues, for example, the prostate, a gamma ray
emitting radionuclide such as indium-113, indium-115 or
technetium-99 can be linked to a prostate homing
20 molecule and, following administration to a subject,
can be visualized using a solid scintillation detector.
Alternatively, for organs or tissues at or near the
external surface of a subject, for example, retina, a
fluorescein-labeled retina homing molecule can be used
25 such that the endothelial structure of the retina can
be visualized using an opthalamoscope and the
appropriate optical system.

   Molecules that selectively home to a
pathological lesion in an organ or tissue similarly can
30 be linked to an appropriate detectable agent such that
the size and distribution of the lesion can be
visualized. For example, where an organ or tissue

homing molecule homes to a normal organ or tissue, but
not to a pathological lesion in the organ or tissue,
the presence of the pathological lesion can be detected
by identifying an abnormal or atypical image of the
5   organ or tissue, for example, the absence of the
detectable agent in the region of the lesion.

A detectable agent also can be an agent that
facilitates detection *in vitro*. For example, a
conjugate comprising a homing molecule linked to an
10   enzyme, which produces a visible signal when an
appropriate substrate is present, can detect the
presence of an organ or tissue to which the homing
molecule is directed. Such a conjugate, which can
comprise, for example, alkaline phosphatase or
15   luciferase or the like, can be useful in a method such
as immunohistochemistry. Such a conjugate also can be
used to detect the presence of a target molecule, to
which the organ homing molecule binds, in a sample, for
example, during purification of the target molecule.

20   In another embodiment, a moiety can be a
therapeutic agent. Thus, the invention provides a
conjugate comprising a lung, skin, pancreas, retina,
prostate, ovary, lymph node, adrenal gland, liver or
gut homing molecule linked to a therapeutic agent.

25   A therapeutic agent can be any biologically
useful agent that, when linked to an organ homing
molecule of the invention, exerts its function at the
site of the selected organ or tissue. For example, a
therapeutic agent can be a small organic molecule that,
30   upon binding to a target cell due to the linked organ
homing molecule, is internalized by the cell where it

can effect its function. A therapeutic agent can be a nucleic acid molecule that encodes a protein involved in stimulating or inhibiting cell survival, cell proliferation or cell death, as desired, in the

5   selected organ or tissue. For example, a nucleic acid molecule encoding a protein such as Bcl-2, which inhibits apoptosis, can be used to promote cell survival, whereas a nucleic acid molecule encoding a protein such as Bax, which stimulates apoptosis, can be

10 used to promote cell death of a target cell.

A particularly useful therapeutic agent that stimulates cell death is ricin, which, when linked to an organ homing molecule of the invention, can be useful for treating a hyperproliferative disorder, for

15 example, cancer. A conjugate comprising an organ homing molecule of the invention and an antibiotic, such as ampicillin or an antiviral agent such as ribavirin, for example, can be useful for treating a bacterial or viral infection in a selected organ or

20 tissue.

A therapeutic agent also can inhibit or promote the production or activity of a biological molecule, the expression or deficiency of which is associated with the pathology. Thus, a protease

25 inhibitor can be a therapeutic agent that, when linked to an organ homing molecule, can inhibit protease activity at the selected organ or tissue, for example, the pancreas. A gene or functional equivalent thereof such as a cDNA, which can replenish or restore

30 production of a protein in a selected organ or tissue, also can be a therapeutic agent useful for ameliorating the severity of a pathology. A therapeutic agent also

can be an antisense nucleic acid molecule, the
expression of which inhibits production of a
deleterious protein, or can be a nucleic acid molecule
encoding a dominant negative protein or a fragment
5   thereof, which can inhibit the activity of a
deleterious protein.

In another embodiment, the invention provides
a conjugate comprising a lung, skin, pancreas, retina,
prostate, ovary, lymph node, adrenal gland, liver or
10   gut homing molecule linked to a tag. A tag can be, for
example, an insoluble support such as a chromatography
matrix, or a molecule such as biotin, hemagglutinin
antigen, polyhistidine, T7 or other molecules known in
the art. Such a conjugate comprising a tag can be
15   useful to isolate a target molecule, to which the organ
homing molecule binds.

When administered to a subject, a conjugate
comprising an organ homing molecule and a moiety is
administered as a pharmaceutical composition
20   containing, for example, the conjugate and a
pharmaceutically acceptable carrier. Pharmaceutically
acceptable carriers are well known in the art and
include, for example, aqueous solutions such as water
or physiologically buffered saline or other solvents or
25   vehicles such as glycols, glycerol, oils such as olive
oil or injectable organic esters.

A pharmaceutically acceptable carrier can
contain physiologically acceptable compounds that act,
for example, to stabilize or to increase the absorption
30   of the complex. Such physiologically acceptable
compounds include, for example, carbohydrates, such as

glucose, sucrose or dextrans, antioxidants, such as
ascorbic acid or glutathione, chelating agents, low
molecular weight proteins or other stabilizers or
excipients. One skilled in the art would know that the
5 choice of a pharmaceutically acceptable carrier,
including a physiologically acceptable compound,
depends, for example, on the route of administration of
the composition. The pharmaceutical composition also
can contain an agent such as a cancer therapeutic agent
10 or other therapeutic agent as desired.

One skilled in the art would know that a
pharmaceutical composition containing an organ homing
molecule can be administered to a subject by various
routes including, for example, orally or parenterally,
15 such as intravenously. The composition can be
administered by injection or by intubation. The
pharmaceutical composition also can be an organ homing
molecule linked to a moiety such as a liposome or other
polymer matrix, which can have incorporated therein,
20 for example, a drug that promotes or inhibits cell
death (Gregoriadis, Liposome Technology, Vol. 1
(CRC Press, Boca Raton, FL 1984), which is
incorporated herein by reference). Liposomes, for
example, which consist of phospholipids or other
25 lipids, are nontoxic, physiologically acceptable and
metabolizable carriers that are relatively simple to
make and administer.

In performing a diagnostic or therapeutic
method as disclosed herein, an effective amount of a
30 conjugate comprising an organ homing molecule must be
administered to the subject. An "effective amount" is
the amount of the conjugate that produces a desired

effect. An effective amount will depend, for example,
on the moiety linked to the organ homing molecule and
on the intended use. For example, a lesser amount of a
radiolabeled homing molecule can be required for
5   imaging as compared to the amount of the radiolabeled
molecule administered for therapeutic purposes, where
cell killing is desired. An effective amount of a
particular conjugate for a specific purpose can be
determined using methods well known to those in the
10   art.

     The route of administration of an organ
molecule will depend, in part, on the chemical
structure of the organ homing molecule. Peptides, for
example, are not particularly useful when administered
15   orally because they can be degraded in the digestive
tract. However, methods for chemically modifying
peptides to render them less susceptible to degradation
by endogenous proteases or more absorbable through the
alimentary tract are well known (see, for example,
20   Blondelle et al., *supra*, 1995; Ecker and Crooke, *supra*,
1995; Goodman and Ro, *supra*, 1995). Such methods can
be performed on peptides that home to a selected organ
or tissue. In addition, methods for preparing
libraries of peptide analogs such as peptides
25   containing D-amino acids; peptidomimetics consisting of
organic molecules that mimic the structure of a
peptide; or peptoids such as vinylogous peptoids, have
been previously described above and can be used to
identify homing molecules suitable for oral
30   administration to a subject.

     The invention provides methods of identifying
a selected organ or tissue by administering to a

subject a conjugate comprising a lung, skin, pancreas,
retina, prostate, ovary, lymph node, adrenal gland,
liver or gut and a detectable agent. A conjugate
comprising an organ homing molecule of the invention
5   linked to a detectable moiety conjugate can be
administered to a subject and used to identify or
visualize a selected organ or tissue. The ability to
visualize an organ, particularly an internal organ,
provides a means diagnose a pathology of the selected
10 organ or tissue. For example, a prostate homing
molecule linked to indium-113 can be administered to a
subject in order to image the prostate. Such a method
can be particularly valuable because methods for
imaging the prostate are limited. The presence of a
15 prostate pathology can be revealed by detecting that a
region of the prostate does not contain the conjugate,
thus indicating an abnormality in circulation to the
region, or by detecting that the prostate is abnormally
enlarged or lacking its normal boundaries. For organs
20 or tissues such as retina, which can be visualized
directly using an ophthalmoscope, a conjugate
comprising a retina homing molecule linked to
fluorescein can be administered to a subject and used
to examine the vascular integrity and circulation in
25 the retina. The absence of a normal or typical pattern
of retinal image can indicate the presence of a retinal
pathology in the region. For example, an abnormal
retinal pattern can reflect vascular changes indicative
of a hyperproliferative or degenerative pathology.

30   In principle, an organ homing molecule of the
invention can have an inherent biological property,
such that administration of the molecule provides
direct biological effect. For example, an organ homing

molecule can be sufficiently similar to a naturally
occurring ligand for the target molecule that the organ
homing molecule mimics the activity of the natural
ligand. Such an organ homing molecule can be useful as
5 a therapeutic agent having the activity of the natural
ligand. For example, where the organ homing molecule
mimics the activity of a growth factor that binds a
receptor expressed by the selected organ or tissue,
such as a skin homing molecule that mimics the activity
10 of epidermal growth factor, administration of the organ
homing molecule can result in cell proliferation in the
organ or tissue. Such inherent biological activity of
an organ homing molecule of the invention can be
identified by contacting the cells of the selected
15 organ or tissue with the homing molecule and examining
the cells for evidence of a biological effect, for
example, cell proliferation or, where the inherent
activity is a toxic effect, cell death.

    In addition, an organ homing molecule of the
20 invention can have an inherent activity of binding a
particular target molecule such that a corresponding
ligand cannot bind the receptor. It is known, for
example, that various types of cancer cells metastasize
to specific organs or tissues, indicating that the
25 cancer cells express a ligand that binds a target
molecule in the organ to which it metastasizes. Thus,
administration of a lung homing molecule, for example,
to a subject having a tumor that metastasizes to lung,
can provide a means to prevent the potentially
30 metastatic cancer cell from becoming established in the
lung. In general, however, the organ homing molecules
of the invention are particularly useful for targeting
a moiety to a selected organ or tissue, particularly to

lung, skin, pancreas, retina, prostate, ovary, lymph
node, adrenal gland, liver or gut. Thus, the invention
provides methods of treating a pathology in a selected
organ or tissue by administering to a subject having
5   the pathology a conjugate comprising an organ homing
molecule of the invention linked to a therapeutic
agent.

Specific disorders of the lung, for example,
can be treated by administering to a subject a
10 conjugate comprising a lung homing molecule linked to a
therapeutic agent. Since a lung homing molecule of the
invention can localize to the capillaries and alveoli
of the lung, disorders associated with these regions
are especially amenable to treatment with a conjugate
15 comprising the lung homing molecule. For example,
bacterial pneumonia often originates in the alveoli and
capillaries of the lung (Rubin and Farber, Pathology
2nd ed., (Lippincott Co.,1994)). Thus, a lung homing
molecule conjugated to a suitable antibiotic can be
20 administered to a subject to treat the pneumonia.
Similarly, cystic fibrosis causes pathological lesions
in the lung due to a defect in the CFTR. Thus,
administration of a lung homing molecule conjugated to
a nucleic acid molecule encoding the CFTR provides a
25 means for directing the nucleic acid molecule to the
lung as an *in vivo* gene therapy treatment method.

The invention also provides methods of
treating a pathology of the skin by administering to a
subject having the pathology a conjugate comprising a
30 skin homing molecule and a therapeutic agent. For
example, a burn victim can be administered a conjugate
comprising a skin homing molecule linked to epithelial

growth factor or platelet derived growth factor such
that the growth factor is localized to the skin where
it can accelerate regeneration or repair of the
epithelium and underlying dermis. Furthermore, a
5 method of the invention can be useful for treating skin
pathologies caused by bacterial infections,
particularly infections that spread through the
hypodermis and dermis or that are localized in these
regions, by administering to a subject a conjugate
10 comprising a skin homing molecule linked to an
antibiotic.

The invention also provides methods of
treating a pathology of the pancreas by administering
to a subject having the pathology a conjugate
15 comprising a pancreas homing molecule linked to a
therapeutic agent. In particular, since a pancreas
homing molecule of the invention can localize to the
exocrine pancreas, a pathology associated with the
exocrine pancreas can be treated and, in some cases,
20 may not adversely affect the endocrine pancreas. A
method of the invention can be particularly useful to
treat acute pancreatitis, which is an inflammatory
condition of the exocrine pancreas caused by secreted
proteases damaging the organ. A conjugate comprising a
25 pancreas homing molecule linked to a protease inhibitor
can be used to inhibit the protease mediated
destruction of the tissue, thus reducing the severity
of the pathology. Appropriate protease inhibitors
useful in such a conjugate are those that inhibit
30 enzymes associated with pancreatitis, including, for
example, inhibitors of trypsin, chymotrypsin, elastase,
carboxypeptidase and pancreatic lipase. A method of
the invention also can be used to treat a subject

having a pancreatic cancer, for example, ductal adenocarcinoma, by administering to the subject a conjugate comprising a therapeutic agent linked to a molecule that homes to pancreas.

5   The methods of the invention also can be used to treat a pathology of the eye, particularly the retina, by administering to a subject having the pathology a conjugate comprising a retina homing molecule linked to a therapeutic agent. For example,
10   proliferative retinopathy is associated with neovascularization of the retina in response to retinal ischemia due, for example, to diabetes. Thus, administration of a conjugate comprising a retina homing molecule linked to a gene that stimulates
15   apoptosis, for example, Bax, can be used to treat the proliferative retinopathy. Similarly, methods of the invention can be used to diagnose or treat prostate, ovary, lymph node, adrenal gland, liver, or gut pathology using the appropriate organ or tissue homing
20   molecules disclosed herein either alone, or linked to a desired moiety.

          An organ or tissue homing molecule is useful, for example, for targeting a therapeutic or detectable agent to the selected organ or tissue. In addition, an
25   organ or tissue homing molecule can be used to identify the presence of a target molecule in a sample. As used herein, the term "sample" is used in its broadest sense to mean a cell, tissue, organ or portion thereof that is isolated from the body. A sample can be, for
30   example, a histologic section or a specimen obtained by biopsy or cells that are placed in or adapted to tissue culture. If desired, a sample can be processed, for

example, by homogenization, which can be an initial
step for isolating the target molecule to which an
organ or tissue homing molecule binds.

5   An organ homing molecule obtained as
disclosed herein can be useful for identifying the
presence of a target molecule, particularly a cell
surface protein, that is recognized by the homing
molecule, or for substantially isolating the target
molecule. Thus, the invention provides methods of
10   identifying target molecules that selectively bind a
lung homing molecule, a skin homing molecule, a
pancreas homing molecule, a retina homing molecule, a
prostate homing molecule, an ovary homing molecule, a
lymph node homing molecule, an adrenal gland homing
15   molecule, a liver homing molecule or a gut homing
molecule. Such a method comprises contacting a sample
of the selected organ or tissue, for example, prostate,
with a prostate homing molecule, and detecting
selective binding of a component of a sample, wherein
20   such binding identifies the presence of a target
molecule.

An organ or tissue homing molecule such as a
prostate homing peptide can be linked to a tag, for
example, a solid support such as a chromatography
25   matrix. The immobilized organ homing molecule then can
be used for affinity chromatography by passing an
appropriately processed sample of prostate tissue over
a column containing the matrix under conditions that
allow specific binding of the prostate homing molecule
30   to the particular target molecule (see, for example,
Deutshcer, Meth. Enzymol., Guide to Protein
Purification (Academic Press, Inc., ed. M.P. Deutscher,

1990), Vol. 182, which is incorporated herein by reference; see, for example, pages 357-379). Unbound and nonspecifically bound material can be removed and the target molecule, which forms a complex with the

5 prostate homing molecule, can be eluted from the column and collected in a substantially isolated form. The substantially isolated prostate target molecule then can be characterized using well known methods. An organ or tissue homing molecule also can be linked to a

10 detectable agent such as a radionuclide, a fluorescent molecule, an enzyme or a labeled biotin tag and can be used, for example, to screen a sample in order to detect the presence of the target molecule or to follow the target molecule during its isolation.

15 As an alternative to using an organ or tissue sample to identify a target molecule of the selected organ or tissue, extracts of cultured cells derived from the selected organ or tissue, or extracts of cultured endothelial cells can be used as the starting

20 material. Selection of cells containing the target molecule can be determined by using binding and cell attachment assays (see Barry et al., Nature Med. 2:299-305 1996), which is incorporated herein by reference). Those cells containing the target molecule

25 can be used to prepare extracts for the isolation and identification of a target molecule, as described above.

Upon identifying an appropriate cell line expressing the target molecule, the target molecule can

30 be labeled by growing the cells in medium containing radiolabeled amino acids. The radiolabeled amino acids are incorporated into the target molecule, thus

facilitating its identification during purification.
Labeled cells then can be extracted with octylglucoside
and the extract can be fractionated by affinity
chromatography using a pancreas homing molecule coupled
5   to a matrix such as Sepharose. Extracts prepared, for
example, from human umbilical vein endothelial cells
can be used as a control. The purified target molecule
then can be microsequenced and antibodies can be
prepared. If desired, oligonucleotide probes can be
10 prepared and used to isolate cDNA clones encoding the
target receptor. Alternatively, an anti-receptor
antibody can be used to isolate a cDNA clone from an
expression library (see Argraves et al., J. Cell Biol.
105:1183-1190 (1987), which is incorporated herein by
15 reference).

        In addition to biochemically isolating a
target molecule, a nucleic acid encoding the target
molecule can be isolated by using, for example, a
pancreas homing molecule as a chemical probe to screen
20 a pancreatic cDNA expression library for clones that
express the target molecule. For example, bacteria
expressing a pancreatic cDNA library can be attached to
a membrane, lysed, and screened with a pancreas homing
molecule conjugated, for example, to an enzyme that
25 produces a colorimetric or fluorescent signal.
Bacterial clones expressing a target molecule are
identified and the cDNA encoding the target molecule
can be isolated. Additionally, a mammalian cell
expression cloning system such as the COS cell system
30 can be used to identify a target molecule. For
example, a cDNA library can be prepared using mRNA from
primary pancreas cells which can be cloned into an
expression vector. Cells expressing a cDNA encoding

the target molecule then can be selected using the pancreas homing peptide as a probe, for example, by panning of cell clones against pancreas homing peptide attached to a plate. Alternatively, phage can be used
5   to display the pancreas homing peptide and can be attached to magnetic beads coated, for example, with anti-M13 antibodies (Pharmacia). Cells expressing the target molecule that bind to the pancreas homing peptide then can be recovered and the plasmids encoding
10 the receptor can be isolated. The recovered plasmid preparations can be divided into pools and examined in COS cell transfections. The procedure can be repeated until single plasmids are obtained that enable the COS cells to bind the pancreas homing peptide.

15   The present invention also provides a method of identifying a MDP-binding homing molecule that selectively homes to lung endothelium. The method includes the steps of contacting membrane dipeptidase (MDP) with one or more molecules; and determining
20 specific binding of a molecule to the MDP, where the presence of specific binding identifies the molecule as a MDP-binding homing molecule that selectively homes to lung endothelium. In a method of the invention, the membrane dipeptidase can be substantially purified and
25 can be, for example, immobilized to a support. The membrane dipeptidase can be any mammalian MDP, for example, human MDP having SEQ ID NO: 448.

As disclosed herein, the CGFECVRQCPERC (SEQ ID NO: 1) peptide can selectively bind to mouse lung
30 vasculature after intravenous injection (Example IIA). Furthermore, as disclosed in Example IVB, a 55 kDa lung cell surface protein that selectively binds GFE-1

(CGFECVRQCPERC; SEQ ID NO: 1) was isolated from rat
lung extracts using affinity chromatography. Tryptic
digestion and sequencing by mass spectrometry revealed
that two peptides derived from the 55 kDa protein were
5   completely identical to portions of rat membrane
dipeptidase (EC 3.4.13.19). Further experimentation
demonstrated that GFE-1 (SEQ ID NO: 1) affinity
purified fractions of rat lung cell extracts have
membrane dipeptidase activity, as indicated by the
10   time-dependent conversion of the specific MDP substrate
Gly-D-Phe to D-Phe (Example IVC and Figure 5).
Furthermore, binding of GFE-1 phage (CGFECVRQCPERC; SEQ
ID NO: 1) and, to a lesser extent, GFE-2 phage
(CGFELETC; SEQ ID NO: 2) to COS cells transfected with
15   membrane dipeptidase was significantly higher than the
binding of phage bearing an unrelated peptide sequence
(Example IVD and Figure 6B), indicating that membrane
dipeptidase is the GFE-1 (SEQ ID NO: 1) receptor.

Thus, as disclosed herein, the lung
20   metalloprotease, membrane dipeptidase, serves as the
receptor for the selective homing of molecules to lung
endothelium. An exemplary class of molecules that
selectively home to lung endothelium by targeting
membrane dipeptidase is the class of peptides bearing a
25   GFE motif, for example, CGFECVRQCPERC (SEQ ID NO: 1).

Membrane dipeptidase, also known as renal
dipeptidase, microsomal dipeptidase,
dehydropeptidase-1, or MDP and currently classified as
EC 3.4.13.19 (previously EC 3.4.13.11), is a plasma
30   membrane glycosyl phosphatidylinositol-anchored
glycoprotein (Keynan et al., in Hooper (Ed.) <u>Zinc</u>
<u>Metalloproteases in Health and Disease</u> Taylor and

Francis, London pages 285-309 (1996), which is
incorporated herein by reference). This zinc
metalloprotease, which is expressed mainly in lung and
kidney brush border, is involved *in vivo* in renal
5 metabolism of glutathione and in pulmonary metabolism
of peptidyl leukotrienes. In addition, MDP is the only
known example of a mammalian β-lactamase. MDP forms a
disulfide-linked homodimer, with the molecular weight
of the monomer ranging from about 48 to 59 kDa
10 depending on the species of origin (Keynan et al.,
Biochem. 35:12511-12517 (1996), which is herein
incorporated by reference; see, also, Example IVB).

Membrane dipeptidase expression has been
detected in several tissues although it is expressed
15 mainly in lung and kidney. There have been reports of
low levels of MDP activity in total extracts from
liver, spleen, small intestine and brain, while others
have found no detectable activity in these organs. In
the mouse, four distinct MDP mRNAs are present, and
20 they are differentially expressed in several organs
(Habib et al., J. Biol. Chem. 271:16273-16280 (1996)).
Organ-specific differences in the nature and extent of
pig MDP N-linked glycosylation also have been reported
(Hooper et al., Biochem. J. 324:151-157 (1997)).

25 In the kidney, MDP expression is restricted
to epithelial cells in the brush border region of the
proximal tubules. In the lung, MDP expression has been
detected in many cell types including endothelial cells
as well as epithelial cells of the conducting airways,
30 alveolar ducts, capillaries, and the basement membrane
of alveoli and terminal bronchioles (Habib et al.,
supra, 1996); Inamura et al., Prostaglandins

Leukotrienes and Essential Fatty Acids 50:85-92
(1994)). MDP expression also has been observed on
endothelial cells of submucosal microvessels in the
human trachea (Yamaya et al., Resp. Physiol.
5   111:101-109 (1998)). The level of MDP activity is
highest in lung (Hirota et al., Eur. J. Biochem.
160:521-525 (1986); Habib et al., Proc. Natl. Acad.
Sci. USA 95:4859-4863 (1998)). This expression pattern
correlates with the strong lung homing of molecules
10 such as GFE-1 (SEQ ID NO: 1).

As used herein, the term "membrane
dipeptidase" is synonymous with "MDP" and refers to the
enzyme currently classified as EC 3.4.13.19 (previously
EC 3.4.13.11) and also known as renal or microsomal
15 dipeptidase or dehydropeptidase-1. The term membrane
dipeptidase encompasses any mammalian membrane
dipeptidase, for example, the human, pig, mouse, rat
and rabbit homologs having the amino acid sequences
shown as SEQ ID NOS: 448 to 452 in Figure 9 as well as
20 related polypeptides having substantial amino acid
sequence similarity to one of these polypeptides. Such
related polypeptides will exhibit greater sequence
similarity to SEQ ID NO: 448, 449, 450, 451 or 452 than
to other zinc metalloproteases or peptidases such as
25 dipeptidyl peptidase IV and include alternatively
spliced forms of MDP and isotype variants of the amino
acid sequences shown in Figure 9. Thus, the term MDP
encompasses homologous polypeptides obtained from
different mammalian species as well as other variants
30 and related polypeptides that generally have amino acid
identities of greater than about 65%, preferably
greater than about 70% and more preferably greater than
about 80% or 90% with SEQ ID NO: 448, 449, 450, 451 or

452. A method of the invention preferably uses human membrane dipeptidase (SEQ ID NO: 448).

The term "substantially purified," as used herein in reference to a membrane dipeptidase
5 polypeptide, means that the polypeptide is in a form that is relatively free from contaminating lipids, nucleic acids, unrelated polypeptides and other cellular material normally associated with membrane dipeptidase in a cell.

10 The methods of the invention for identifying a MDP-binding homing molecule can be practiced *in vivo* or *in vitro,* and membrane dipeptidase can be obtained from a number of sources. Sources of membrane dipeptidase include whole cells or cell extracts
15 containing endogenous or exogenous MDP. Additional sources of MDP include partially purified cell extracts; biochemically purified enzyme, for example, affinity purified MDP; recombinant polypeptides; and transfected cell lines.   -

20 Affinity chromatography can be particularly useful for purifying or partially purifying membrane dipeptidase for use in a method of the invention. For example, membrane dipeptidase can be purified from lung cell extracts by affinity chromatography using
25 immobilized GFE-1 peptide (SEQ ID NO: 1) as described for murine and rat membrane dipeptidase in Example IVC. Similarly, membrane dipeptidase can be obtained by affinity chromatography using other immobilized ligands such as cilastatin. For example, membrane dipeptidase
30 can be efficiently purified in two steps, through selective release of MDP by bacterial phosphatidyl

inositol-specific phospholipase C (PI-PLC) coupled with
cilastatin-Sepharose affinity chromatography as
described in Littlewood et al., Biochem. J. 257:361-367
(1989); and Campbell et al., J. Biol. Chem. 259:14586-
5 14590 (1984), each of which is incorporated herein by
reference.

Recombinant membrane dipeptidase also can be
useful in a method of the invention. The amino acid
and nucleic acid sequences of a variety of MDP homologs
10 are known in the art. Nucleic acid sequences encoding
the membrane dipeptidase polypeptides shown in Figure 9
can be obtained, for example, from databases such as
GenBank or from the literature (see, for example,
GenBank Accession Numbers D13139 and 285150; Adachi et
15 al., J. Biol. Chem. 265:3992-3995 (1990); Rached et
al., 1990; Keynan et al., FEBS Letts. 349:50-54 (1994);
Satoh et al., Biochim. Biophys. Acta 1163:234-242
(1993); Adachi et al., Biochim. Biophys. Acta 1132:311-
314 (1992); An et al., Biochim. Biophys. Acta 1226:337-
20 340 (1994); and Igarashi and Karniski, Biochem. J.
280:71-78 (1991), each of which is incorporated herein
by reference). Novel membrane dipeptidase cDNAs can be
isolated from additional mammalian species with a
nucleotide sequence as a probe or primer using methods
25 well known in the art of molecular biology (Innis et
al. (Ed.), PCR Protocols, San Diego: Academic Press,
Inc. (1990); Erlich, *supra,* 1989; Sambrook et al.,
supra, 1989, each of which is incorporated herein by
reference). One skilled in the art knows a variety of
30 methods for expression of MDP encoding nucleic acids
and subsequent isolation of recombinant MDP
polypeptide.

In the methods of the invention for identifying a MDP-binding homing molecule that selectively homes to lung endothelium, specific binding of a molecule to MDP identifies the molecule as a

5   MDP-binding homing molecule that selectively homes to lung endothelium. The term "specific binding," as used herein in reference to a molecule and MDP, means that the molecule has an affinity for MDP that is measurably different from a non-specific interaction. Specific

10 binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity, for example, a peptide of similar size that lacks a GFE

15 motif. In this case, specific binding is indicated if the molecule has measurably higher affinity for membrane dipeptidase than the control molecule. Specificity of binding also can be determined, for example, by competition with a control molecule that is

20 known to bind to MDP, for example, a peptide containing the GFE motif.

The term specific binding, as used herein, includes both low and high affinity specific binding. Specific binding can be exhibited, for example, by a

25 low affinity MDP-binding homing molecule having a Kd for membrane dipeptidase of about 10^{-4} M to about 10^{-7} M. Specific binding also can be exhibited by a high affinity MDP-binding homing molecule, for example, a MDP-binding homing molecule having a Kd for membrane

30 dipeptidase of at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, at least about 10^{-10} M, or at least about 10^{-11} M or 10^{-12} M or greater. A MDP-binding homing peptide including the sequence

X_1-G-F-E-X_2 (SEQ ID NO: 17), where X_1 and X_2 each is 1 to 10 independently selected amino acids, can have, for example, a Kd for membrane dipeptidase of about 2 x 10^{-5} M to 10^{-7} M, for example, a Kd of about 10^{-6} to

5   10^{-7} M. Both low and high affinity MDP-binding homing molecules that selectively home to lung endothelium can be useful in selectively directing a moiety to lung endothelium in a subject as described further below.

A variety of art known techniques can be used
10 to determine specific binding of a molecule to membrane dipeptidase according to a method of the invention. Conditions suitable for specific binding are described, for example, in Example IVB. Specific binding also can be determined by transfecting cells lacking MDP
15 expression with MDP as described, for example, in Example IVD. In this case, specific binding is determined, in part, by significantly higher binding of a molecule to the MDP-transfected cells than to untransfected cells.

20   The present invention is directed to the surprising discovery that MDP-binding molecules home specifically to the lung vasculature in spite of MDP expression in other tissues such as kidney. As disclosed herein, injection of MDP-binding GFE-1 (SEQ
25 ID NO: 1) bearing phage into the mouse circulation resulted in rapid binding of the phage to lung microvasculature with some diffuse staining on neighboring cells. The same results were obtained by injecting GFE-1 (SEQ ID NO: 1) bearing phage into rat
30 circulation. In particular, the MDP-binding GFE-1 phage did not bind, for example, to the brush border of kidney proximal tubules, which expresses high levels of

MDP. These results indicate that expression of MDP on the luminal surface of lung endothelial cells can mediate homing of MDP-binding phage from the circulation to lung endothelium, while MDP-binding

5 phage cannot access and home to kidney MDP. Thus, MDP mediates selective homing of molecules to lung endothelium in preference to other endothelial cells.

Selective homing of GFE-1 (SEQ ID NO: 1) bearing phage to lung vasculature further demonstrates

10 that a moiety such as a phage can be linked to a MDP-binding homing molecule and thereby selectively directed to lung endothelium. Thus, the present invention provides methods of selectively targeting moieties, such as phage, gene therapy vectors or

15 antibiotics, to lung endothelium for treatment of lung disorders.

A method of the invention for selectively directing a moiety to lung endothelium in a subject involves administering to the subject a conjugate

20 containing a moiety linked to a MDP-binding homing molecule that selectively homes to lung endothelium, whereby the moiety is selectively directed to lung endothelium in the subject. The MDP-binding homing molecule is identified by contacting membrane

25 dipeptidase (MDP) with one or more molecules; and determining specific binding of a molecule to the MDP, where the presence of specific binding identifies the molecule as a MDP-binding homing molecule that selectively homes to lung endothelium. A method of the

30 invention can be useful for targeting genes or medications to the lung in a subject suffering, for example, from pneumonia; asthma; emphysema; respiratory

infection; chronic bronchitis; chronic interstitial
lung disease; lung cancer; pleurisy or cystic fibrosis.
If desired, a method of the invention can be used
prophylactically, for example, to selectively direct a
5 moiety to the lung endothelium of an individual with a
family history of a lung disorder, for example, or an
individual susceptible to lung infection.

A moiety to be selectively directed to lung
endothelium can be a physical, chemical or biological
10 material such as a virus, viral gene therapy vector,
cell, liposome, microcapsule, micropump or other
chambered microdevice, which can be used, for example,
as a drug delivery system. Such microdevices generally
are biologically inert and, if desired, can be
15 biodegradable or excretable. Various moieties,
including microcapsules, which can contain an agent,
and methods for linking a moiety or chambered
microdevice to an organic molecule are well known in
the art and commercially available as described
20 hereinabove. Exemplary moieties that can be linked to
a MDP-binding homing molecule that selectively homes to
lung endothelium to produce a therapeutic conjugate
include therapeutic antimicrobial bacteriophage;
antibiotics such as ampicillin; and antiviral agents
25 such as ribavirin (see above).

A moiety to be selectively directed to lung
endothelium according to a method of the invention can
be, for example, a therapeutic bacteriophage ("phage").
Phage have been shown to be nontoxic (Ochs et al., _J._
30 _Clin. Invest._ 50:2559-2568 (1971), which is
incorporated herein by reference), and the use of phage
therapy is known in the art for treatment of bacterial

infections such as antibiotic-resistant infections
(Barrow and Soothill, Trends in Microbiology 5:268-271
(1997); Slopek et al., Arch. Immunol. Ther. Exp.
35:553-561 (1987); and Merrill et al., Proc. Natl.
5   Acad. Sci., USA 93:3188-3192 (1996), each of which is
incorporated herein by reference; see, also, Practical
Applications of Bacteriophages CRC Press, Boca Raton,
Florida). For example, in a series of 550 patients,
the majority of whom had been unsuccessfully treated
10   with antibiotic therapy, phage therapy resulted in 75-
100% success in ameliorating a variety of infections
including respiratory tract suppurative infections and
broncopneumonia (Slopek et al., *supra,* 1987). Phage
therapy can be particularly useful in treating
15   nosocomial and multi-drug-resistant infections.

        Studies have shown that both prophylaxis and
treatment are possible using fewer phage than
inoculating bacteria, indicating that phage multiply *in
vivo.* Thus, phage therapy can be more effective than
20   conventional treatment with antibiotics, such as
streptomycin, tetracycline, ampicillin and
sulfafurazole, because of the ability of phage to
replicate *in vivo.* If desired, treatment with a phage
conjugate containing a MDP-binding homing molecule can
25   be combined with antibiotic drug therapy.

        Preferably, a phage moiety to be selectively
directed to lung endothelium is a lytic phage. Such a
phage can be readily modified by standard genetic
techniques to encode a MDP-binding homing peptide such
30   as GFE-1 (SEQ ID NO: 1) to produce a conjugate that is
selectively directed to lung endothelium. Phage useful
in the methods of the invention include, for example,

T4-related phage, also known as members of the "T-Even family of phages." One skilled in the art understands that a phage moiety is selected with a receptor specificity for the bacteria characteristic of an
5   infection to be treated, for example, phage with receptor specificity for *Staphylococcus, Klebsiella, Proteus, Escherichia, Shigella, Pseudomonas* or *Salmonella.* If desired, the pathogenic bacteria can be typed and monitored for phage sensitivity. For
10 treatment of resistant bacteria, a phage conjugate can include, if desired, a mixture of phage with different receptor specificities against a variety of types of bacteria.

A phage/MDP-binding homing conjugate can be
15 administered to a subject, for example, intravenously. Such a conjugate also can be administered orally, for example, as 10 ml sterile phage lysate half an hour before each meal, with gastric juices neutralized by Vichy water, baking soda or gelatin. After oral
20 administration, phage/MDP-binding homing conjugates access the blood and subsequently are selectively directed to lung endothelium. Administration can be repeated, if needed, daily over a period of several weeks or months. An appropriate dose of a phage
25 conjugate can readily be determined by the skilled person and generally will be in the range of about 100 to about 10^{10} plaque forming units (pfu), usually from about 100 to 10^6 pfu.

A moiety to be selectively directed to lung
30 endothelium for gene therapy can be a gene therapy vector. As used herein, the term "gene therapy vector" means a vector containing a nucleic acid component,

which, when delivered to host cells, transiently or permanently expresses an encoded gene product in the host cells *in vivo*.

A variety of gene therapy vectors that can be
5   selectively directed to lung endothelium using an
MDP-binding homing molecule also are known in the art,
including viral and non-viral vectors, for example,
retroviral vectors, adenoviral vectors,
adeno-associated vectors (AAV), herpesvirus vectors and
10   liposome plasmid vectors (Chang, Somatic Gene Therapy
CRC Press, Boca Raton, Florida (1995), each of which is
incorporated herein by reference). Retroviral and AAV
vectors can be useful, for example, for permanent
expression, while adenovirus, herpesvirus and
15   liposome-plasmid vectors generally give transient
expression. Adenoviral vectors, for example, have been
used to express the cystic fibrosis transmembrane
receptor (CFTR) and recombinant α1-antitrypsin in lung
(Rosenfeld et al., Cell 68:143 (1992); Rosenfeld et
20   al., Science 252: 431 (1991), each of which is
incorporated herein by reference). Liposome DNA
complexes also have been used to effect gene transfer
to the lung (see, for example, Zhu et al., Science
261:209 (1993), which is incorporated herein by
25   reference). Phage vectors also can be useful for
expressing a desired nucleic acid *in vivo* (see, for
example, Ivanenkov et al., Biochimica et Biophysica
Acta 1448:450-462 (1999); Ivanekov et al., Biochimica
et Biophysica Acta 1448:463-472 (1999), each of which
30   is incorporated by reference herein).

The methods of the invention for selectively
directing a moiety to lung endothelium using a

conjugate containing a MDP-binding homing molecule such
as GFE-1 (SEQ ID NO: 1) can be useful in the
therapeutic management of a variety of pulmonary
disorders. For example, by selectively directing a
5   gene therapy vector encoding a cytokine to lung
endothelium, the methods of the invention can be useful
for immunotherapy. A variety of cytokines or
chemokines can be useful in stimulating an immune
response, such as an anti-cancer or anti-viral immune
10 response, when administered according to a method of
the invention. Such cytokines and chemokines include
GM-CSF, G-CSF, IFN-γ, IFN-α, TNF-α, TNF-β, IL-1. IL-2,
IL-3, IL-4, IL-6, IL-7, IL-10, IL-12, lymphotactin or
DC-CK1 (Pardoll, Annu. Rev. Immunol. 13:399-415 (1995;
15 Hunt et al., J. Immunotherapy 14:314-321 (1993); Chang,
supra, 1995, each of which is incorporated herein by
reference). The methods of the invention can be more
effective than administration of recombinant cytokines,
due to the short half-life of cytokines in the
20 circulation and the lack of their targeting to lung.

     As discussed above, the methods of the
invention can be useful for treating pulmonary
infections by selectively directing a phage moiety or
an antibiotic drug such as streptomycin, tetracycline,
25 ampicillin or sulfafurazole to lung endothelium. For
example, the methods of the invention can be useful for
treating infections secondary to acquired
immunodeficiency syndrome (AIDS) or cystic fibrosis.

     The methods of the invention for selectively
30 directing a moiety to lung endothelium also can be used
for replacement gene therapy of lung disorders such as
α1-antitrypsin deficiency and cystic fibrosis (Alton

and Geddes, <u>Brit. J. Hosp. Medicine</u> 58:38-40 (1997);
Wood, <u>Radiology</u> 204:1-10 (1997), each of which is
incorporated herein by reference). Genes encoding wild
type α1-antitrypsin (α1-AT) and the cystic fibrosis
5   transmembrane receptor (CFTR) have been isolated
(Riordan et al., <u>Science</u> 245:1066-1073 (1989); Rich et
al., <u>Nature</u> 347:358-63 (1990; Rosenfeld et al., <u>Science</u>
252:431-434 (1991), each of which is incorporated
herein by reference) and can be transferred selectively
10 to the lung in a gene therapy vector linked to a
MDP-binding homing molecule such as GFE-1 (SEQ ID
NO: 1).

       In one embodiment, the invention provides a
method for selectively directing a moiety to lung
15 endothelium where the MDP-binding homing molecule is a
peptide including the sequence X_1-G-F-E-X_2 (SEQ ID NO:
17), where X_1 and X_2 each is 1 to 10 independently
selected amino acids. Such a MDP-binding homing
peptide can include, for example, the sequence
20 CGFECVRQCPERC (SEQ ID NO: 1) or CGFELETC (SEQ ID
NO: 2).

       In another embodiment, the invention provides
a method for selectively directing a moiety to lung
endothelium where the MDP-binding homing molecule
25 contains the following Structure 1:

where R^2 and R^3 are hydrocarbon radicals in the range respectively of 3-10 and 1-15 carbon atoms; in either one of these R^2 or R^3 hydrocarbon chains 1-6 hydrogens may be

5   replaced by halogens or a nonterminal methylene may be replaced by oxygen or sulfur, including oxidized forms of the latter; additionally, a terminal hydrogen in R^3 can also be replaced by hydroxyl or thiol, which

10 may be acylated or carbamoylated; or the hydrogen can be replaced by amino, which may be derivatized as in an acylamino, ureido, amidino, quanidino, or alkyl or substituted amino group, including quaternary nitrogen

15 grouping; or, there may be replacement by acid groups such as carboxylic, phosphonic or sulfonic acid groups or esters or amides thereof, or cyano; or combinations thereof, such as a terminal amino acid grouping; and R^1

20 is hydrogen or lower alkyl (C_{1-6}) or dialkylaminoalkyl, or a pharmaceutically acceptable cation. Such an MDP-binding homing molecule can be, for example, 7-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropane

25 carboxamido)-2-heptenoic acid, also known as cilastatin.

        An MDP-binding homing molecule can be, for example, a compound having Structure 1 in which R^2 is branched alkyl or cycloalkyl

with a limitation that the carbon adjacent to the carbonyl cannot be tertiary.

An MDP-binding homing molecule also can be, for example, a compound having
5   Structure 1 in which R^2 is branched alkyl or cycloalkyl with a limitation that the carbon adjacent to the carbonyl cannot be tertiary, and in which R^3 is n-alkyl (1-9 carbons) or n-alkyl (1-9 carbons) having a terminal
10   substituent which is a quaternary nitrogen, amine derivative or amino acid derived group. An MDP-binding homing molecule can be, for example, a compound having Structure 1 in which R^2 is 2,2-dimethylcyclopropyl or
15   2,2-dichlorocyclopropyl and in which R^3 is a hydrocarbon chain of 3 to 7 carbon atoms without a terminal substituent or having a terminal substituent which is trimethylammonium, amidino, guanidino or
20   2-amino-2-carboethylthio.

Exemplary MDP-binding homing molecules having Structure 1 useful in the invention include the following: Z-2-(2,2-dimethylcyclopropane carboxamido)-8-
25   trimethylammonium hydroxide-2-octenoic acid inner salt; Z-2-(2,2-dichlorocyclo propanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt; Z-2-(2,2-dimethylcyclopropane carboxamido)-8-
30   guanidino-2-octenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-

guanidino-2-octenoic acid; Z-2-(2,2-
dimethylcyclopropanecarboxamido)-8-ureido-2-
octenoic acid; Z-8-(1-2-amino-2-carboxy
ethylthio)-2-(2,2-dimethylcyclopropane
5   carboxamido)-2-octenoic acid; Z-2-(2,2-
dimethylcyclopropane carboxamido)-2-octenoic
acid (racemic and dextrorotatory forms); Z-2-
(2,2-dichloro cyclopropanecarboxamido)-2-
octenoic acid;7-(L-2-amino-2-carboxyethylthio)
10 -2-(2,2-dimethylcyclopropane carboxamido)-2-
heptenoic acid; and 6-(L-2-amino-2-
carboxyethylthio)-2-(2,2-dimethylcyclopropane
carboxamido)-2-hexenoic acid.

        As set forth above, the methods of the
15 invention for targeting treatment to the lungs can be
practiced with an MDP-binding homing molecule, which is
a Z-2-acylamino- 3-monosubstituted propenoate having
Structure 1,

$$R^3 \diagdown \overset{\displaystyle C}{\underset{\displaystyle \|}{}} \diagup H$$

$$R^2CONH \diagup \overset{\displaystyle C}{} \diagdown COOR^1$$
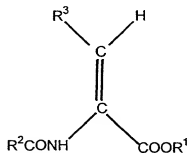
20   where R^2 and R^3 are hydrocarbon radicals in
the range respectively of 3-10 and 1-15 carbon atoms.
In either of these hydrocarbon radicals R^2 and R^3, up
to 6 hydrogens may be replaced by halogens, or a non-
terminal methylene may be replaced by oxygen or sulfur,
25 including oxidized forms of the latter.

A terminal hydrogen in R^3 also can be replaced by a hydroxyl or thiol group, which may be acylated, such as with an alkanoyl acid of 1-8 carbon atoms, or carbamoylated, including alkyl and dialkyl carbamate derivatives; or the hydrogen can be replaced by an amino group, which may be derivatized as in an acylamino, ureido, amidino, guanidino, or alkyl or substituted alkyl amino group, including quaternary nitrogen groupings; or, alternatively, there may be replacement by acid groups such as carboxylic, phosphonic or sulfonic acid groups or esters or amides thereof, as well as cyano; or combinations thereof, such as a terminal amino acid grouping.

In an MDP-binding homing molecule having Structure 1, R^2 is preferably a branched alkyl or cycloalkyl radical (C_{3-10}), with a limitation that the carbon adjacent to the carbonyl cannot be tertiary. R^2 cannot be phenyl or straight chain lower alkyl of 1-4 carbon atoms, where R^3 is straight chain lower alkyl of 1-4 carbon atoms. R^1 is hydrogen, loweralkyl (C_1-C_6) or dialkylaminoalkyl (e.g., $-CH_2CH_2N(C_2H_5)_2$,$-CH_2CH(CH_3)N(CH_3)_2$.

An MDP-binding homing molecule having Structure 1 above can have an asymmetric form. Racemic Z-2-(2,2 dimethylcyclopropane carboxamido)-2-octenoic acid has been resolved. Activity resides in the dextrorotatory isomer, which has the S-configuration.

Within the definition of R^2 of a compound having Structure 1, the following sub-groups are included:

              -R4

wherein R^4 is a straight, branched, or cyclic hydrocarbon radical of 3-10 carbon atoms which may be substituted

5   as specified above in the definition of R^2;

              $-R^5R^6$

wherein R^5 is cycloalkyl of 3-6 carbon atoms and R^6 is either 1 or 2 alkyl substituents which may be joined to form another ring on the cycloalkyl group, or R^5 and R^6

10 may be substituted as specified above in the definition of R^2; and

              $-R^7R^8$

wherein R^7 is an alkylene group of 1-3 carbon atoms and R^8 is cycloalkyl of 3-6 carbon atoms which may be

15 substituted as specified above in the definitions of R^2 and R^3.

      Particularly preferred substituents within the definition of R^2 in Structure 1 include the 2,2-dimethylcyclopropyl and the 2,2-dichlorocyclopropyl

20 groups.

      Within the definition of R^3 in Structure 1, particularly preferred groups of compounds include n-alkyl (1-9 carbons) and n-alkyl (1-9 carbons) having a terminal substituent which is a quaternary nitrogen,

25 amine derivative, or amino acid derived group.

      The term "quaternary nitrogen" is used herein in reference to Structure 1 to mean a tetrasubstituted or heteroaromatic nitrogen which is positively charged. An ammonium moiety, substituted with hydrocarbon groups

having 1-7 carbon atoms, which can be the same or different, is signified.

As used herein in reference to Structure 1, the term "amino derivative" means a group such as
5   amino, acylamino, ureido, amidino, guanidino and alkyl (1-7 carbon atoms) derivatives thereof.

As used herein in reference to Structure 1, the term "amino acid derived group" means a moiety such as cysteinyl ($-SCH_2CH(NH_2)COOH$) or sarcosyl ($-$
10   $N(CH_3)CH_2COOH$) in which a hydrogen joined to O, N or S of known amino acids is replaced.

Particularly preferred MDP-binding homing molecules having Structure 1 are those in which R^2 is 2,2-dimethyl cyclopropyl or dichlorocyclopropyl, and R^3
15   is a hydrocarbon chain of 3 to 7 carbon atoms without a terminal substituent, or having a terminal substituent which is trimethylammonium, amidino, guanidino, or 2-amino-2-carboxyethylthio.

Exemplary MDP-binding homing molecules useful
20   in the invention include Z-2-(2,2-dimethylcyclopropane carboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt; Z-2-(2,2-dichlorocyclopropane carboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt; Z-2-(2,2-dimethylcyclopropane
25   carboxamido)-8-formamidino-2-octenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid; Z-2-(2,2-dimethylcyclopropane carboxamido)-8-ureido-2-octenoic acid; Z-8-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethyl
30   cyclopropanecarboxamido)-2-octenoic acid; Z-2-(2,2-

dimethylcyclopropanecarboxamido)-2-octenoic acid
(racemic and dextrorotatory forms); Z-2-(2,2-
dichlorocyclopropanecarboxamido)-2-octenoic acid; 7-(L-
2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropane
5 carboxamido)-2-heptenoic acid; and 6-(L-2-amino-2-
carboxyethylthio)-2-(2,2-dimethylcyclopropane
carboxamido)-2-hexenoic acid.

Methods for preparing an MDP-binding homing
molecule having Structure 1 are disclosed herein and
10 known in the art. Several methods for preparing such
an MDP-binding homing molecule are set forth in Example
VI (see, also, U.S. Patent No. 4,616,038 to Kahan et
al., which is incorporated herein by reference).

Additional MDP-binding homing molecules known
15 in the art also can be used to selectively target a
gene or medication to lung endothelium according to a
method of the invention. Such MDP-binding homing
molecules include substituted 2-alkenoic acids
including the following Structure 2:

20

$$Y—S—(CH_2)_n$$
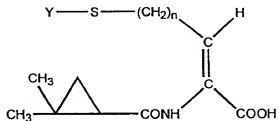
wherein n is an integer from 3 to 5 and Y is a
heterocyclic or phenyl group that may be substituted or
unsubstituted, and the lower alkyl (C_1-_6) esters and
pharmaceutically acceptable salts thereof.

As used herein in reference to Structure 2, the term "heterocyclic" means pyridyl, pyrimidinyl, tetrazolyl, imidazolyl, thiazolinyl and the like. Such heterocyclic rings and phenyl rings can be

5　unsubstituted or substituted with hydroxyl, oxo, carbonyl, or methyl. Y-groups in Structure 2 include, for example, 2-pyridinyl; 4-pyridinyl; 3-hydroxy-2-pyridinyl; 3-carboxy-2-pyridinyl; 5-carboxy-2-pyridinyl; 2-carboxyphenyl;

10　1-methyl-1,2,3,4-tetrazol-5-yl; and 4-carboxy-6-hydroxy-2-pyrimidinyl.

The configuration at the cyclopropyl center of a substituted 2-alkenoic acid MDP-binding homing molecule having Structure 2 preferably is S (+),

15　although an R,S (+/-) racemic mixture of the compound also can be used in a method of the invention.

Particularly active MDP-binding homing molecules of this class are those in which n is 4 and Y is 3-carboxy-2-pyridyl or 3-hydroxy-2-pyridyl, each in

20　the S-form. Exemplary MDP-binding homing molecules having Structure 2 include: Z-7-(3-hydroxy-2-pyridylthio)-2-(2,2-dimethylcyclopropane carboxyamido)-2-heptenoic acid and Z-(5-carboxy-2-pyridylthio)-2-(2,2-dimethylcyclopropanecarboxyamido)-

25　2-heptenoic acid.

An MDP-binding homing molecule having Structure 2, as well as various pharmaceutically acceptable derivatives such as alkali and alkaline earth metal, ammonium or amine salts, or the like,

30　which are also useful in the invention, can be prepared by the skilled person using routine methods. An

MDP-binding homing molecule having Structure 2 can be prepared, for example, by condensation of a bromoalkenoic acid with the appropriate mercaptan, YSH, in water in the presence of sodium bicarbonate at
5   ambient temperature as described, for example, in U.S. Patent No. 4,406,902 to Ashton et al., which is incorporated herein by reference.

An MDP-binding homing molecule useful in the invention also can be a phosphinic acid having the
10 following Structure 3:

$$H_2N\text{---}CH(R_1)\text{---}\overset{\displaystyle O}{\underset{\displaystyle OH}{P}}\text{---}CH_2\text{---}CH(R_2)\text{---}CO_2H$$

or the following Structure 4:

$$H_2N\text{---}CH(R_1)\text{---}\overset{\displaystyle O}{\underset{\displaystyle OH}{P}}\text{---}CH_2\text{---}C(\text{=}CHR_5)\text{---}CO_2H$$
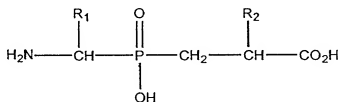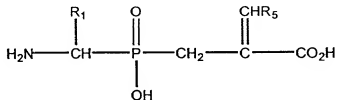
where:
15 R_1 is
    (a) $C_2\text{-}C_{12}$ linear or branched unsubstituted alkyl;
    (b) $C_2\text{-}C_{12}$ linear or branched substituted alkyl;
    (c) $C_2\text{-}C_{12}$ linear or branched monoalkenyl;
    (d) $C_2\text{-}C_{12}$ linear or branched alkynyl;
20 (e) $C_7\text{-}C_{20}$ aralkyl, wherein the alkyl chain is linear or branched $C_1\text{-}C_8$ and the aryl moiety is $C_6\text{-}C_{12}$;

(f) C_3-C_7 cycloalkyl;

(g) C_4-C_{10} cycloalkylalkyl, for structure 4 only;
where the above values for R_1, excluding (a), can
be substituted by one or more: C_1-C_4 alkoxy, C_6-C_{12}
5 aryloxy, C_1-C_4 alkylthio, C_6-C_{12} arylthio, C_3-C_6
cycloalkyloxy, C_3-C_6 cycloalkylthio, C_7-C_{10} aralkyloxy,
C_7-C_{16} aralkylthio;

R_2 is

(a) H or C_1-C_{12} linear or branched alkyl;

10 (b) C_2-C_{12} linear or branched monoalkenyl;

(c) C_7-C_{20} aralkyl, wherein the alkyl chain is linear or
branched C_1-C_8 and the aryl moiety is C_6-C_{12};

(d) heterocyclic alkyl, wherein the alkyl chain is
linear or branched C_1-C_8 and the heterocyclic ring
15 is 5-6 membered, optionally fused with a benzene
ring, fully aromatic, containing 1-2: O, N or S
heteroatoms;

(e) C_3-C_7 cycloalkyl;

(f) C_4-C_{10} cycloalkylalkyl;

20   where the above values for R_2 can be
substituted by one or more: halo, hydroxy, carboxy, C_1-
C_4 alkoxycarbonyl, C_7-C_{16} arylalkoxycarbonyl, C_3-C_7
cycloalkyl, C_1-C_4 alkoxy, C_6-C_{12} aryloxy, C_3-C_6
cycloalkyloxy, C_3-C_6 cycloalkylthio, amino, mono- or
25 di-C_1-C_8 alkylamino, thio, C_1-C_4 alkylthio, C_6-C_{12}
arylthio, C_7-C_{16} aralkylthio, or the radical $-S-(CH_2)_n-$
$CH(NH_2)COOH$;

R^5 is

(a) H or C_1-C_{12} linear or branched alkyl;

30 (b) C_2-C_{12} linear or branched monoalkenyl;

(c) C_7-C_{20} aralkyl, wherein the alkyl chain is linear or
branched C_1-C_8 and the aryl moiety is C_6-C_{12};

(d) heterocyclic alkyl, wherein the alkyl chain is linear or branched C_1-C_8 and the heterocyclic ring is 5-6 membered, optionally fused with a benzene ring, fully aromatic, containing 1-2: O, N or S heteroatoms;

5   (e) C_4-C_{10} cycloalkylalkyl;

(f) C_3-C_7 cycloalkyl;

where the above value for R_5 can be substituted by one or more: halo, hydroxy, carboxy, C_1-C_4 alkoxycarbonyl, C_7-C_{16} arylalkoxycarbonyl, C_3-C_7

10 cycloalkyl, C_1-C_4 alkoxy, C_6-C_{12} aryloxy, C_3-C_6 cycloalkyloxy, C_3-C_6 cycloalkylthio, amino, mono-or di-C_1-C_8 alkylamino, thio, C_1-C_4 alkylthio, C_6-C_{12} arylthio, C_7-C_{16} aralkylthio, or the radical $-S-(CH_2)_n-CH(NH_2)COOH$; and including MDP-binding stereoisomers

15 and racemates thereof Structures 3 and 4.

In an MDP-binding homing molecule having Structure 3 or 4, the values for R_1 for (a) C_2-C_{12} linear or branched unsubstituted alkyl include ethyl, propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-

20 butyl, n-pentyl, isopentyl, n-hexyl, n-heptyl, n-octyl, iso-octyl, n-decyl, n-undecyl, n-dodecyl and the like. Preferred in this series is n-butyl, isobutyl, n-pentyl and n-hexyl.

In an MDP-binding homing molecule having

25 Structure 3 or 4, values for R_1 for (b) C_1-C_{12} linear or branched alkyl, where substituted, include the values above for R_1(a), substituted by the above-defined substituents, including the following preferred substituents: methoxy, ethoxy, propoxy, butoxy,

30 methylthio, ethylthio, propylthio, butylthio, cyclopentyloxy, cyclopentylthio, cyclopropylthio,

benzylthio, 2-phenethylthio, 2-phenethylthio and the like.

In an MDP-binding homing molecule having Structure 3 or 4, values for R_1 for (e) C_2-C_2 linear or branched monoalkenyl include ethenyl, propenyl, 1-butenyl, 2-butenyl, 1-pentenyl, 2-pentenyl, 2-methyl-2-butenyl and the like.

In an MDP-binding homing molecule having Structure 3 or 4, values for R_1 for (d) C_2-C_{12} linear or branched alkynyl include: ethynyl, propynyl, 1-butynyl, 2-butynyl, 2-methyl-3-pentynyl and the like.

Values for R_1 for (d) C_2-C_{12} linear or branched alkynyl include: ethynyl, propynyl, 1-butynyl, 2-butynyl, 2-methyl-3-pentynyl and the like.

Values for R_1 for (e) C_7-C_{20} aralkyl include benzyl, 2-phenylethyl, 1-phenylethyl, 4-methylphenyl-methyl and the like. Preferred in this series is benzyl.

Values for R_1 for (f) C_4-C_{10} cycloalkylalkyl include: cyclohexylmethyl, cyclopentylmethyl, 2-cyclohexylethyl, 2-cycloctylethyl, and the like. Preferred in this series is cyclopentylmethyl and cyclohexylmethyl.

Values for R_1 for (g) C_3-C_7 cycloalkyl include: cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like. Preferred in this series is cyclopentyl and cyclohexyl.

Preferred substituent values for R_1 include: methoxy, ethoxy, phenoxy, methylthio, ethylthio, phenylthio, benzyloxy, 2-phenylethyloxy, benxylthio, 2-phenylethylthio, and the like.

5   The values of the alkyl, alkenyl groups for R_2 and R_5, except where noted otherwise, represented by any of the variables include linear or branched, alkyl and monoalkenyl and chain hydrocarbon radicals from two to twelve carbon atoms, for example, ethyl, n-propyl, 10   isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl, isopentyl, n-heptyl, n-nonyl, 4,4-dimethylpentyl, or vinyl, allyl, 1-butenyl, 2-butenyl, 5-hexenyl and the like. Preferred are isopropyl, n-butyl, n-pentyl, n-heptyl or 1-butenyl.

15   Values of C_3-C_7 cycloalkyl and C_4-C_{10} cycloalkylalkyl include: cyclopentyl, cyclohexyl, cyclopentyl-methyl, cyclopentylethyl, cyclohexylmethyl, cyclohexylethyl, cyclopropyl, and the like.

The aralkyl group represented by the above 20   variables has from one to eight carbon atoms in the alkyl portion and "aryl" where noted, represents phenyl, naphthyl, or biphenyl. Representative examples include benzyl, phenethyl, 4-phenyl-n-butyl, 1-phenyl-n-octyl, and the like.

25   In an MDP-binding homing molecule of the invention having Structure 3 or 4, the aromatic heterocyclic, i.e. "heteroaryl" substituent, are synonymous, and recited above represents a 5- or 6-membered aromatic ring containing from one to three O, 30   N or S heteroatoms, preferably one O or S or 1-3N

heteroatoms, such as for example, pyridyl, thienyl, furyl, imidazolyl, and thiazolyl as well as any bicyclic group derivable therefrom in which any of the above heterocyclic rings is fused to a benzene ring
5   such as, for example, indolyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzothiazolyl, benzofuryl, and benzothienyl.

The named substituents on the R_2 and R_5 alkyl and alkenyl chains can be present on the aromatic rings
10   in the aralkyl, heterocyclic alkyl and heteroaryl groupings as well. The site of substitution can be any available sites and the substitution can involve one or more of the same or different groups.

The substituents are: halo, meaning fluoro,
15   chloro, bromo or iodo; hydroxy; carboxy; C_1-C_4 linear or branched alkoxycarboxy, e.g. methoxycarbonyl and ethoxycabonyl; C_7-C_{16} arylalkoxy carbonyl, e.g. benzyloxycarbonyl, n-butyloxyoarbonyl; C_3-C_7 cycloalkyl, e.g. cyclopentyl and cyclohexyl; C_1-C_4
20   alkoxy, e.g. t-butoxy and ethoxy; C_6-C_{12} aryloxy, e.g. biphenyloxy, benzyloxy; amino; mono- or di-C_1-C_8 dialkylamino, e.g. methylamino, isopropylamino, n-butylamino, isohexylamino, N,N-diethylamino, methylethylamino, methyl-t-butylamino, di-n-octylamino;
25   thio; C_1-C_4 alkylthio, e.g. methylthio, ethylthio C_6-C_{12} arylthio, e.g. phenylthio; C_7-C_{16} aralkylthio, e.g. benzylthio, naphthyl-methylthio; the radicals $-S-CH_2-CH(NH_2)COOH$ and $-S-(CH_2)_2-CH(NH_2)COOH$, both preferably in the L-configuration; and, where a thio substituent
30   is present, R_2 or R_5 must be at least a C_2 alkyl grouping. Where an aryl or heteroaryl group is present in the substituent, the ring carbons can additionally

be substituted by one or more of linear or branched C_1-C_4 alkyl, e.g. methyl, ethyl, isopropyl, t-butyl; trihalomethyl, "halo" having the same meaning as described above, e.g. trichloromethyl, trifluoromethyl;

5 nitro, cyano or sulfonamide.

Preferred are the compounds wherein:
R_1 is cyclohexylmethyl, cyclopentyhnethyl, n-pentyl, n-butyl, n-hexyl, isobutyl, R_2 and R_5 are:
C_3-C_7 cycloalkyl; C_1-C_{10} linear or branched alkyl,

10 substituted or unsubstituted; C_7-C_{14} aralkyl, substituted or unsubstituted. Wherein these groups can be substituted with halo, amino, mono- or di-C_1-C_4 linear or branched alkylamino, carboxyl, C_1-C_4 alkoxycarbonyl, hydroxy, C_1-C_4 alkoxy, C_5-C_6 cycloalkyl,

15 C_6-C_{10} aryroxy, thio, C_1-C_4 linear or branched alkylthio, C_6-C_{10} arylthio, C_7-C_{14} aralkylthio, $-S-(CH_2-)_n-$ $CH(NH_2)CO_2H$; wherein the aryl group ring carbons can further be substituted by linear or branched C_1-C_4 alkyl; R_3 and R_4 are hydrogen, C_1-C_4 linear or branched

20 alkyl e.g. methyl, ethyl, or C_7-C_{14} aralkyl e.g. benzyl.

In an MDP-binding homing molecule having Structure 3 or 4, the carbon preferably is attached to R_1 in the (R) or (RS) configuration, more preferably (R), and the carbon attached to R_2 is in the (R), (RS)

25 or (S) configuration, preferably (RS) or (S) and if R_5 is present, the double bond preferably is in the Z configuration. One skilled in the art understands that an MDP-binding homing molecule based on Structure 3 can be used in the form of salts derived from inorganic or

30 organic acids and bases.

Methods for preparing an MDP-binding homing molecule having Structure 3 or Structure 4 are well known in the art. See, for example, Parsons et al., Biochemistry International 23:1107-1115 (1991); and

5   U.S. Patent No. 5,145,990, each of which is incorporated herein by reference.

A variety of other MDP-binding homing molecules known in the art also can be useful for selectively directing a gene or medication to lung

10 endothelium according to a method of the invention. Such MDP-binding homing molecules include those described in Kahan et al., U.S. Patent No. 4,616,038; Ashton et al., U.S. Patent No. 4,406,902; Parsons et al., U.S. Patent No. 5,145,990; Parsons et al.,

15 Biochem. International 23:1107-1115 (1991); Uchida et al., U.S. Patent No. 5,061,730; Hashimoto et al., J. Antibiotics XLIII:281-285 (1990); and Takase et al., J. Antibiotics XLIII:38-42 (1990), each of which is incorporated herein by reference.

20   Metastatic cells display an altered repertoire of cell adhesion molecules, allowing escape from the primary tumor, adhesion and penetration of the extracellular matrix and entry into the microvasculature. Most such cells are destroyed by

25 geometric and hemodynamic forces in their first encounter with the narrow capillary net, usually in the lungs. Although the frequency of metastasis to the lungs has been attributed solely to mechanical entrapment of tumor cell emboli, it has long been

30 observed that certain tumor cell types prefer to metastasize to specific target organs.

Several lines of evidence indicate that the selection of a target organ for metastasis is mediated by specific interactions between blood-born cancer cells and the endothelium of that target organ

5   (Albelda, Lab. Invest. 68: 4-17 (1993); Auerbach et al., Cancer Res. 47:1492-1496 (1987); Johnson et al., Cancer Res. 51:394-399 (1991), each of which is incorporated herein by reference). In the lung, two vascular receptors have been found to mediate adhesion

10 of metastatic cells. LuECAM-1, an endothelial surface protein with sequence homology to chloride channels, mediates adhesion of malignant melanoma cells to lung endothelium (Elble et al., J. Biol. Chem. 272:27853-27861 (1997), which is incorporated herein by

15 reference). Furthermore, a protease, lung endothelial dipeptidylpeptidase IV (DPP IV/CD 26), promotes homing of metastatic breast and prostate carcinoma cells to lung (Johnson et al., J. Cell Biol. 121: 1423-1432 (1993), which is incorporated herein by reference).

20 Fibronectin present on the surface of the metastatic cells was shown to be the ligand for DPP IV-dependent homing of the breast cancer cells to lung vasculature (Chen et al., J. Biol. Chem. 273:24207-24215 (1998), which is incorporated herein by reference). These

25 results indicate that organ-selective homing can be mediated by classical cell adhesion molecules as well as other molecules.

As disclosed herein, selective homing of GFE-1 (SEQ ID NO: 1) to lung endothelium *in vivo* is

30 mediated by the cell surface protease, MDP. As further disclosed herein, administration of GFE-1 (SEQ ID NO: 1) can inhibit experimental lung metastasis of two melanoma cell lines (human C8161 and mouse B16) in

mice, demonstrating that GFE-1 (SEQ ID NO; 1) can compete with metastasizing tumor cells for binding to the same receptor (see Figure 8). These results indicate that MDP serves as a receptor for

5 metastasizing tumor cells on lung vasculature and that an MDP-binding homing molecule can reduce or prevent the anchoring of lung metastases.

Thus, the present invention provides a method of reducing or preventing lung metastasis in a subject

10 having cancer by administering to the subject a membrane dipeptidase (MDP)-binding homing molecule. Primary tumors that commonly spread to the lungs include breast, colorectal, lung, testicular, pancreatic, esophageal, stomach, ovarian, renal cell

15 and prostate carcinomas, osteogenic and soft tissue sarcomas and melanoma (Smith, Seminars Oncology Nurs. 14:178-186 (1998), which is incorporated herein by reference). The methods of the invention can be used, for example, to reduce or prevent lung metastasis of

20 breast cancer, kidney cancer, melanoma, bladder cancer, cancer of the cervix, ovarian cancer, prostate cancer, colorectal cancer or lung cancer.

As used herein, the term "metastasis" means the transfer of malignant cells from one site to

25 another site not directly connected with it, after which the cells form a proliferative focus. Thus, metastatic cells become detached from a primary tumor, move to a different part of the body and grow as a separate mass of tumor. One skilled in the art

30 understands that a malignant cell can travel locally from a first site within an organ to a second site within the same organ or can travel distantly within

the body to a different organ. Furthermore, malignant cells of a metastasis can themselves give rise to additional metastases.

The term "lung metastasis," as used herein, refers to the transfer of malignant cells to one or more sites within lung not directly connected with the first site, after which the cells form a proliferative focus within the lung. The resulting detached masses of cancer cells within the lung are termed lung "metastases" or secondary tumors. Lung metastases can originate from a variety of primary cancers, which they generally will resemble histologically. Breast cancer, kidney cancer and melanoma, for example, frequently metastasize to lung. In addition, cancers of the bladder, cervix, ovary and prostate metastasize to lung and, less frequently, colorectal cancers or primary lung cancers metastasize to one or more secondary sites in lung.

The term "reducing or preventing," as used herein in reference to lung metastasis, means that the rate or extent of lung metastasis is diminished. Thus, lung metastasis is reduced or prevented where the development of lung metastasis is completely precluded or is significantly delayed; or where the size or number of lung metastases is significantly diminished. One skilled in the art understands that a delay in development of lung metastases or a decrease in the size or number of lung metastases is measured relative to the rate or extent of lung metastases in one or more control subjects not treated with an MDP-binding homing molecule according to a method of the invention.

If addition, a subject not having detectable cancer can be treated prophylactically with an MDP-binding homing molecule to reduce or prevent the future occurrence of lung metastases. Such a subject
5   can be, for example, suspected of having cancer or can have a family history of cancer.

As set forth above, an MDP-binding homing molecule exhibits selective homing to lung endothelium and is characterized, in part, by exhibiting specific
10 binding to membrane dipeptidase. An MDP-binding homing molecule useful in reducing or preventing lung metastasis can readily be identified by specific binding to MDP as described hereinabove. If desired, additional MDP-binding homing molecule also can be
15 identified by the ability to competitively inhibit binding of known MDP-binding peptides, for example, GFE-1 (SEQ ID NO: 1) or cells, for example, C8161 cells to membrane dipeptidase.

In one embodiment, an MDP-binding homing
20 molecule useful in reducing or preventing lung metastasis is a lung homing peptide including the sequence X_1-G-F-E-X_2 (SEQ ID NO: 17), where X_1 and X_2 each is 1 to 10 independently selected amino acids, such as a peptide including the sequence CGFECVRQCPERC
25 (SEQ ID NO: 1) or CGFELETC (SEQ ID NO: 2). Such an MDP-binding homing molecule can be, for example, the peptide CGFECVRQCPERC (SEQ ID NO: 1) or CGFELETC (SEQ ID NO: 2). Additional MDP-binding homing molecules containing the sequence X_1-G-F-E-X_2 (SEQ ID NO: 17),
30 where X_1 and X_2 each is 1 to 10 independently selected amino acids, can be identified as described below, for

example, by screening a combinatorial peptide library
which includes the motif GFE as invariant residues.

In another embodiment, an MDP-binding homing
molecule is a membrane dipeptidase inhibitor. As used
5   herein, the term "membrane dipeptidase inhibitor" is
synonymous with "MDP inhibitor" and means an organic
molecule that selectively decreases the enzymatic
activity of membrane dipeptidase. In general, an MDP
inhibitor is a molecule that binds to the active site
10   of MDP. An MDP inhibitor can be an organic molecule
such as a drug; peptide; modified peptide or peptide
mimetic; protein or protein fragment; nucleic acid
molecule such as a ribonucleic or deoxyribonucleic
acid; oligosaccharide; lipid; glycolipid; or
15   lipoprotein. Exemplary MDP inhibitors disclosed herein
are CGFECVRQCPERC (SEQ ID NO: 1) and cilastatin.

The term "selectively inhibits," as used
herein in reference to an MDP inhibitor, means that the
inhibitor decreases MDP activity in a manner that is
20   selective for the MDP enzyme as compared to related but
different enzymes such as other proteases. Thus, an
MDP inhibitor is distinct from a non-specific inhibitor
of, for example, zinc metalloproteases. Thus, an MDP
inhibitor can selectively decrease MDP activity while
25   having little or no effect on the activity of, for
example, dipeptidyl peptidase IV.

Assays for measuring MDP enzymatic activity
are known in the art. MDP cleaves dipeptide substrates
in which the N-terminal amino acid is in the
30   L-configuration and is unblocked. The C-terminal amino
acid is either in the L- or D-configuration, with the

enzyme hydrolyzing substrates with a D-configuration C-terminal residue more rapidly. MDP activity can be assayed, for example, using glycyl-D-phenylalanine (Gly-D-Phe) as a substrate (Keynan et al., *supra,* 1996;
5 Parsons et al., *supra,* 1991). A convenient specific fluorimetric assay for MDP enzymatic activity uses Gly-D-Phe as a substrate and subsequent reaction of D-amino acid oxidase with the released D-Phe (see Example IVE; see, also, Heywood and Hooper, <u>Analyt.</u>
10 <u>Biochem.</u> 226:10-14 (1995), which is incorporated herein by reference).

A membrane dipeptidase inhibitor can be a molecule that exhibits structural homology to a natural MDP substrate. For example, following cleavage of the
15 tripeptide glutathione by γ-glutamyl transpeptidase to form glutamate and cysteinylglycine (Cys-Gly), the dipeptide Cys-Gly is recognized and cleaved by MDP, which cleaves only dipeptides. The amino acid sequence of glutathione is similar to the N-terminal portion of
20 GFE-1 (SEQ ID NO: 1), in which the first two amino acids are Cys-Gly. As shown in Figure 7, GFE-1 (SEQ ID NO: 1) inhibits hydrolysis of the Gly-D-Phe substrate in a dose-dependent manner, indicating that GFE containing peptides such as GFE-1 (SEQ ID NO: 1) can be
25 MDP inhibitors. These results further indicate that an MDP inhibitor can be structurally similar to a naturally occurring MDP substrate.

A variety of MDP inhibitors are known in the art. For example, an MDP inhibitor can be an
30 MDP-binding homing molecule such as an MDP-binding molecule having Structure 1, Structure 2, Structure 3 or Structure 4, described hereinabove.

An MDP inhibitor useful in the invention can have, for example, a Ki for membrane dipeptidase of about 10^{-4} M to about 10^{-12} M. For example, a MDP inhibitor including the sequence X_1-G-F-E-X_2 (SEQ ID

5 NO: 17), where X_1 and X_2 each is 1 to 10 independently selected amino acids, can have, for example, a Ki for membrane dipeptidase of about 2×10^{-5} M to 10^{-7} M, for example, a Ki of about 10^{-6} to 10^{-7} M.

An MDP inhibitor useful in the invention can

10 exhibit, for example, a Ki against MDP of 1000 nM or less. An MDP inhibitor useful in reducing or preventing lung metastasis also can exhibit, for example, a Ki against MDP of 100 nM or less or a Ki of 1 nM or less. For example, an MDP inhibitor having

15 Structure 3 or Structure 4 can be a tight binding inhibitor with a Ki from, for example, about 0.5 nM to 10 nM. MDP inhibitors having Structure 3 or Structure 4 therefore can be particularly useful in the methods of the invention for reducing or preventing lung

20 metastasis by administering an MDP inhibitor to a subject having cancer.

In another preferred embodiment, an MDP inhibitor comprises Structure 1, where R^2 and R^3 are hydrocarbon radicals in the range respectively of 3-10

25 and 1-15 carbon atoms; in either one of these R^2 or R^3 hydrocarbon chains 1-6 hydrogens may be replaced by halogens or a nonterminal methylene may be replaced by oxygen or sulfur, including oxidized forms of the latter; additionally, a terminal hydrogen in R^3 can

30 also be replaced by hydroxyl or thiol, which may be acylated or carbamoylated; or the hydrogen can be replaced by amino, which may be derivatized as in an

acylamino, ureido, amidino, quanidino, or alkyl or
substituted amino group, including quaternary nitrogen
grouping; or, there may be replacement by acid groups
such as carboxylic, phosphonic or sulfonic acid groups
5   or esters or amides thereof, or cyano; or combinations
thereof, such as a terminal amino acid grouping; and R^1
is hydrogen or lower alkyl (C_{1-6}) or dialkylaminoalkyl,
or a pharmaceutically acceptable cation. Such an MDP
inhibitor for reducing or preventing lung metastasis
10 can be, for example, 7-(L-2-amino-2-carboxyethylthio)-
2-(2,2-dimethylcyclopropane carboxamido)-2-heptenoic
acid, also known as cilastatin.

An MDP inhibitor can be, for example, a
compound having Structure 1 in which R^2 is branched
15 alkyl or cycloalkyl with a limitation that the carbon
adjacent to the carbonyl cannot be tertiary.

An MDP inhibitor also can be, for example, a
compound having Structure 1 in which R^2 is branched
alkyl or cycloalkyl with a limitation that the carbon
20 adjacent to the carbonyl cannot be tertiary, and in
which R^3 is n-alkyl (1-9 carbons) or n-alkyl (1-9
carbons) having a terminal substituent which is a
quaternary nitrogen, amine derivative or amino acid
derived group. An MDP inhibitor can be, for example, a
25 compound having Structure 1 in which R^2 is 2,2-
dimethylcyclopropyl or 2,2-dichlorocyclopropyl and in
which R^3 is a hydrocarbon chain of 3 to 7 carbon atoms
without a terminal substituent or having a terminal
substituent which is trimethylammonium, amidino,
30 guanidino or 2-amino-2-carboethylthio.

Exemplary MDP inhibitors having Structure 1 useful in the invention include the following: Z-2-(2,2-dimethylcyclopropane carboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt; Z-2-(2,2-dichlorocyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt; Z-2-(2,2-dimethylcyclopropane carboxamido)-8-guanidino-2-octenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-guanidino-2-octenoic acid; Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-ureido-2-octenoic acid; Z-8-(l2-amino-2-carboxy ethylthio)-2-(2,2-dimethylcyclopropane carboxamido)-2-octenoic acid; Z-2-(2,2-dimethylcyclopropane carboxamido)-2-octenoic acid (racemic and dextrorotatory forms); Z-2-(2,2-dichloro cyclopropanecarboxamido)-2-octenoic acid; 7-(L-2-amino-2-carboxyethylthio) -2-(2,2-dimethylcyclopropane carboxamido)-2-heptenoic acid; and 6-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropane carboxamido)-2-hexenoic acid.

The present invention also provides a method of reducing or preventing lung metastasis in a subject having cancer by administering to the subject a MDP negative regulatory agent. A MDP negative regulatory agent useful in the invention can be, for example, a soluble MDP polypeptide or an antibody that selectively reacts with MDP.

Further provided herein are methods of reducing or preventing cell homing to lung endothelium in a subject by administering to the subject a MDP negative regulatory agent. A MDP negative regulatory agent useful for reducing or preventing cell homing to

lung endothelium can be, for example, a soluble MDP polypeptide or an antibody that selectively reacts with MDP.

As used herein, the term "MDP negative
5   regulatory agent" means an organic molecule that, directly or indirectly, selectively reduces MDP expression or activity. Such MDP negative regulatory agents can be, for example, drugs; nucleic acid molecules, including ribonucleic acid molecules and
10 deoxyribonucleic acid molecules; peptides; variants or modified peptides or peptide mimetics; proteins or fragments thereof; antibodies or fragments thereof; oligosaccharides; lipids; glycolipids; or lipoproteins.

One skilled in the art understands that a MDP
15 negative regulatory agent can act by a variety of mechanisms to selectively reduce MDP expression or activity. An MDP negative regulatory agent can be, for example, an organic molecule that acts to reduce the amount of functional MDP expressed in lung endothelium.
20 Such an agent can selectively reduce MDP transcription or translation and can be, for example, an antisense oligonucleotide, a transcription factor that negatively regulates MDP expression, or a nucleic acid molecule encoding such a transcription factor.

25   An MDP negative regulatory agent also can be, for example, a fragment of MDP that effectively competes with wild type membrane dipeptidase to reduce or prevent metastatic or other cells from selectively homing to MDP in lung endothelium. A soluble,
30 extracellularly expressed form of MDP or other dominant negative fragment of MDP can be a MDP negative

regulatory agent useful in the invention. A MDP regulatory agent also can be an MDP mimic, which is a protein or other organic molecule that shares tertiary structural homology with MDP or a subpart thereof, and

5 which, when expressed, competes with endogenous MDP for binding to metastatic or other homing cells such as lymphocytes. An MDP mimic can structurally resemble the region of MDP that contacts a metastatic cell; an MDP mimic can structurally resemble, for example, the

10 active site of MDP.

In one embodiment, a MDP negative regulatory agent is an antibody that selectively reacts with MDP. As used herein, an antibody that "selectively reacts with MDP" binds with substantially higher affinity to

15 membrane dipeptidase than to an unrelated polypeptide such as another zinc metalloprotease. The term "antibody" is used herein in its broadest sense to include polyclonal and monoclonal antibodies, as well as polypeptide fragments of antibodies that retain a

20 selective affinity for membrane dipeptidase of at least about 1×10^5 M^{-1}. Antibody fragments such as Fab, F(ab')$_2$ and Fv fragments can selectively react with membrane dipeptidase and, therefore, are included within the meaning of the term antibody as defined

25 herein. The term antibody as used herein includes naturally occurring antibodies, as well as non-naturally occurring antibodies and fragments such as chimeric antibodies and humanized antibodies that are selectively reactive with membrane dipeptidase.

30 Methods for producing antibodies are routine in the art. Membrane dipeptidase, which can be prepared from natural sources or produced recombinantly

as described above, or a fragment thereof, such as a
synthetic peptide, can be used as an immunogen. Non-
immunogenic fragments or synthetic peptides can be made
immunogenic by coupling the hapten to a carrier

5   molecule such as bovine serum albumin (BSA) or keyhole
limpet hemocyanin (KLH). In addition, various other
carrier molecules and methods for coupling a hapten to
a carrier molecule are well known in the art as
described, for example, by Harlow and Lane, Antibodies:

10   A Laboratory Manual (Cold Spring Harbor Laboratory
Press, 1988), which is incorporated herein by
reference. Antibodies, including non-naturally
occurring antibodies such as, chimeric and humanized
antibodies, also can be constructed using solid phase

15   peptide synthesis, produced recombinantly or obtained,
for example, by screening combinatorial libraries
consisting of variable heavy chains and variable light
chains as described by Borrebaeck (Ed.), Antibody
Engineering (Second edition) New York: Oxford

20   University Press (1995), which is incorporated herein
by reference.

        In another embodiment, a MDP negative
regulatory agent is a soluble MDP polypeptide. As used
herein, the term "soluble polypeptide" means a

25   polypeptide that is not membrane bound. A soluble MDP
polypeptide useful in the invention is secreted and,
thus, expressed extracellularly.

        A soluble MDP polypeptide useful in the
invention can be, for example, a truncated or mutated

30   MDP polypeptide lacking one or more C-terminal residues
required for GPI anchor addition (see Figure 9). To
determine whether a particular truncated or mutated MDP

derivative is soluble, the derivative can be expressed, for example, in COS cells, and the transfected cells and supernatant subsequently assayed for MDP activity to determine whether the MDP is membrane bound or

5 soluble.

The present invention also provides a method of identifying a molecule that reduces or prevents lung metastasis by contacting membrane dipeptidase (MDP) with one or more molecules; and determining MDP

10 activity in the presence of the molecule as compared to a control value, where diminished MDP activity in the presence of the molecule identifies the molecule as a molecule that reduces or prevents lung metastasis. The membrane dipeptidase can be, for example, substantially

15 purified. MDP activity can be determined, for example, by release of D-Phe from Gly-D-Phe.

In one embodiment of the invention, a molecule that reduces or prevents lung metastasis can be identified by contacting membrane dipeptidase (MDP)

20 with one or more molecules; determining MDP activity in the presence of the molecule as compared to a control value; administering the molecule to a subject having cancer; and assaying lung metastasis in the subject as compared to a control level of metastasis, where

25 diminished MDP activity in the presence of the molecule identifies the molecule as a molecule that reduces or prevents lung metastasis.

The following examples are intended to

30 illustrate but not limit the present invention.

EXAMPLE I

IN VIVO PANNING

This example demonstrates methods for preparing a phage display library and screening the
5 library using *in vivo* panning to identify phage expressing peptides that home to a selected organ or tissue.

A. Preparation of phage libraries:

Phage display libraries were constructed
10 using the fuse 5 vector as described by Koivunen et al., *supra*, 1995; see, also, Koivunen et al., *supra*, 1994b). Libraries encoding peptides designated CX_6C (SEQ ID NO: 26), CX_7C (SEQ ID NO: 24), $CX_{10}C$ (SEQ ID NO: 30)) $CX_3CX_3CX_3C$ (SEQ ID NO: 25), X_2CX_4CX (SEQ ID
15 NO: 23), and X_7 (SEQ ID NO: 29), were prepared, where "C" indicates cysteine and "X_N" indicates the given number of individually selected amino acids. These libraries can display cyclic peptides when at least two cysteine residues are present in the peptide.

20   The libraries containing the defined cysteine residues were generated using oligonucleotides constructed such that "C" was encoded by the codon TGT and "X_N" was encoded by NNK, where "N" is equal molar mixtures of A, C, G and T, and where "K" is equal molar
25 mixtures of G and T. Thus, the peptide represented by CX_6C (SEQ ID NO: 26) can be represented by an oligonucleotide having the sequence $TGT(NNK)_6TGT$ (SEQ ID NO: 31). Oligonucleotides were made double stranded by 3 cycles of PCR amplification, purified and ligated
30 to the nucleic acid encoding the gene III protein in

the fuse 5 vector such that, upon expression, the peptide is present as a fusion protein at the N-terminus of the gene III protein.

5　　The vectors were transfected by electroporation into MC1061 cells. Bacteria were cultured for 24 hr in the presence of 20 µg/ml tetracycline, then phage were collected from the supernatant by precipitation twice using polyethylene glycol. Each library contained about 10^{12} transducing

10　units/ml (TU; individual recombinant phage).

B. *In vivo* panning of phage:

　　For lung and pancreas, a mixture of phage libraries containing 10^{10} TU was diluted in 200 µl DMEM and injected into the tail vein of anesthetized BALB/c

15　mice (2 month old; Harlan Sprague Dawley; San Diego CA); AVERTIN (0.017 ml/g) was used as anesthetic (Pasqualini and Ruoslahti, *supra*, 1996). After 1-4 minutes, mice were snap frozen in liquid nitrogen or, after about 5 minutes of phage circulation, the

20　mice were perfused through the heart with 5-10 ml of DMEM (SIGMA; St. Louis MO). To recover the phage, the organs from the perfused mice or partially thawed organs from snap frozen mice were collected and weighed, then were homogenized in 1 ml DMEM-PI (DMEM

25　containing protease inhibitors (PI); phenylmethyl sulfonyl fluoride (PMSF; 1 mM), aprotinin (20 µg/ml), leupeptin (1 µg/ml)).

　　Organ samples were washed 3 times with ice cold DMEM-PI containing 1% bovine serum albumin (BSA),

30　then directly incubated with 1 ml K91-kan bacteria for

1 hr.   Ten ml NZY medium containing 0.2 µg/ml
tetracycline (NZY/tet) was added to the bacterial
culture, the mixture was incubated in a 37°C shaker for
1 hr, then 200 µl aliquots were plated in agar plates
5 containing 40 µg/ml tetracycline (tet/agar).

        For *in vivo* panning of skin, two month old
BALB/c nude mice were used to avoid contamination by
hair. The mice were injected intravenously with phage
as described above and, after perfusion through the
10 heart, the skin was removed in large sections and
placed on an ice cold plate with the hypodermis facing
up. The skin was scraped with a scalpel to remove
mostly hypodermis, which was then processed for phage
recovery as described below.

15   For *in vivo* panning of retina, two month old
female Simonson Albino rats were used to provide larger
tissue samples than mice. The rats were anesthetized
with phenobarbital (50 mg/kg body weight), and, while
under deep anesthesia, the abdominal cavity of the rats
20 was opened and 10^{10} TU of a phage library was injected
into the left ventricle of the heart through the
diaphragm. After 2-5 minutes of phage circulation, the
eyes were removed, then washed once in 70% EtOH and
once in PBS. The anterior chamber, with cornea and
25 lens, was removed and the retina was peeled from the
remaining posterior chamber. The tissue was weighed,
homogenized with a syringe bulb in 1 ml of ice cold
DMEM containing protease inhibitors (1 mM PMSF,
20 µg/ml aprotinin and 1 µg/ml of leupeptin; all from
30 SIGMA; St. Louis MO). The tissue was washed 3 times
with 1 ml of DMEM and the phage were rescued as
described below.

Approximately 250 to 300 individual bacterial colonies containing phage recovered from the various organs or tissues were grown for 16 hr in 5 ml NZY/tet. In some experiments, approximately 1000 individual

5   bacteria containing phage were picked and the phage were amplified in 2 ml of NZY/tet or the entire plate containing phage was scraped, pooled and grown in bulk and processed for injection. Where phage were cultured separately, the cultures were pooled and the phage were

10 injected into mice or rats as described above for a second round of *in vivo* panning. In some experiments, a third or fourth round of panning was performed. Phage DNA was purified from individual bacterial colonies obtained and the DNA sequences encoding the

15 peptides expressed by selected phage were determined (see Koivunen et al., *supra*, 1994b).

EXAMPLE II

CHARACTERIZATION OF PEPTIDES THAT HOME TO A SELECTED ORGAN

20 This example demonstrates that an organ or tissue homing peptide of the invention selectively homes to a selected organ or tissue including an organ containing a component of the RES.

A. Lung is the selected organ

25 After two or three rounds of *in vivo* panning of mice injected with a cyclic $CX_3CX_3CX_3C$ (SEQ ID NO: 25) or a cyclic CX_6C (SEQ ID NO: 26) phage display library, four peptides that homed to lung were identified. The peptide sequences CGFECVRQCPERC (SEQ

30 ID NO: 1; GFE-1) and CGFELETC (SEQ ID NO: 2; GFE-2)

appeared repeatedly in the lung and two peptide
sequences from the CX_6C (SEQ ID NO: 26) library
CTLRDRNC (SEQ ID NO: 15) and CIGEVEVC (SEQ ID NO: 16)
also were found to home to lung (see Table 2, below).

5   To determine the specificity of lung homing
of the individual peptides identified, phage displaying
the peptides were amplified individually, diluted to
the same input titer and administered to mice.
Following administration, control kidney and brain
10   organ was removed and the number of TU of phage in
lung, kidney and brain was determined. The results
shown in Figure 2 reveal that 10x and 35x more phage
having the peptide sequence CGFECVRQCPERC (SEQ ID
NO: 1; GFE-1) bound to lung than to kidney and brain,
15   respectively. Figure 2 also reveals that CGFELETC (SEQ
ID NO: 2; GFE-2) was found in lung at a 12x and 20x
greater level than in kidney and brain, respectively.
The lung homing peptides CGFECVRQCPERC (SEQ ID NO: 1;
GFE-1), CGFELETC (SEQ ID NO: 2; GFE-2), CTLRDRNC (SEQ
20   ID NO: 15) and CIGEVEVC (SEQ ID NO: 16) are enriched in
lung at 35x, 9x, 6x and 5x, respectively, over
unselected phage (see Figure 2). Thus, substantial
enrichment of phage binding to the lung was observed in
comparison to control brain and kidney and in
25   comparison to unselected phage.

          Specificity for the lung homing peptides was
also determined by competition experiments with
GST-fusion peptides. A GST-GFE-1 (SEQ ID NO: 1) fusion
30   peptide coadministered with GFE-1 (SEQ ID NO: 1)
inhibited GFE-1 (SEQ ID NO: 1) homing to the lung,
whereas GST had no effect on homing (Figure 3A). In
addition, the inhibitory effect of the GST-GFE-1 (SEQ

ID NO: 1) on homing was dose dependent; 70% inhibition of homing occurred when injecting 500 μg of the GST-GFE-1 (SEQ ID NO: 1) fusion protein (Figure 3B). Coinjection of GST-GFE-2 (SEQ ID NO: 2) with GFE-2 (SEQ
5   ID NO: 2) inhibited homing to a lesser extent; 30% inhibition of homing occurred when injecting 500 μg of the GST-GFE-2 (SEQ ID NO: 2) fusion protein (Figure 3B). Interestingly, the GST-GFE-1 (SEQ ID NO: 1) fusion was more efficient at inhibiting GFE-2 (SEQ ID
10 NO: 2) homing to the lung; 60% inhibition of GFE-2 (SEQ ID NO: 2) homing occurred when injecting 500 μg of the GST-GFE-1 (SEQ ID NO: 1) fusion protein (Figure 3B). However, no inhibitory effect of GFE-1 (SEQ ID NO: 1) homing was observed when coinjecting GST-GFE-2 (SEQ ID
15 NO: 2). This can be explained by GFE-1 (SEQ ID NO: 1) having a higher affinity for a shared target molecule than GFE-2 (SEQ ID NO: 2).

.   Additional lung homing peptides were obtained and the amino acid sequences were determined for the
20 inserts (see Table 2). Peptides containing a GFE motif predominated (see Table 1; SEQ ID NOS: 1 and 2). Other peptides that were present more than once in lung are indicated by an asterisk in Table 2 (below), and the remaining peptides were identified one time each.

25   These results indicate that the selection of the peptides containing the GFE motif represents the selective binding of several independent phage displaying peptides having the GFE sequence and is not an artifact due, for example, to phage amplification.
30 In addition, in some cases, phage that expressed peptides having the same amino acid sequence were encoded by oligonucleotides having different sequences,

therefore confirming that homing of a particular phage
to a lung is due to the specific peptide expressed on
the phage.

These results demonstrate that *in vivo*
5   panning can be used to screen phage display libraries
in order to identify phage expressing peptides that
home to lung, which contain a component of the RES.

B.   Skin is the selected tissue:

After two or three rounds of *in vivo* panning
10   of mice injected with a cyclic $CX_3CX_3CX_3C$ (SEQ ID
NO: 25) phage display library, the peptide sequence
CVALCREACGEGC (SEQ ID NO: 3), which appeared repeatedly
in skin, was identified (Table 1). To determine the
specificity of skin homing of the sequence
15   CVALCREACGEGC (SEQ ID NO: 3), phage displaying the
peptide was amplified individually, diluted to the same
input titer and administered to mice. Following
administration, control kidney and brain organ were
removed and the number of TU of phage in skin, kidney
20   and brain was determined.

The results revealed that 7x more phage
displaying the peptide sequence CVALCREACGEGC (SEQ ID
NO: 3) bound to skin than to kidney or brain (see
Figure 2; Table 1). The peptide CVALCREACGEGC (SEQ ID
25   NO: 3) was enriched in skin 7x over unselected phage
(Figure 2). Thus, substantial enrichment of phage
binding to the skin was observed in comparison to
control brain and kidney and in comparison to
unselected phage.

Additional skin homing peptides were obtained by screening the cyclic $CX_3CX_3CX_3C$ (SEQ ID NO: 25) or $CX_{10}C$ (SEQ ID NO: 30) phage display libraries; amino acid sequences were determined for the inserts as shown in Table 5, below. Peptides that were identified more than one time during screening are indicated by an asterisk. For example, 14% of recovered $CX_3CX_3CX_3C$ (SEQ ID NO: 25) phage that homed to head skin had the sequence CVDVCCDGCPVCC (SEQ ID NO: 437). Phage having the sequence RVPLSGDVEH (SEQ ID NO: 438), LRVMSFTSGQ (SEQ ID NO: 439), or RFSVGSLFGS (SEQ ID NO: 440) each constituted 14% of recovered $CX_{10}C$ (SEQ ID NO: 30) phage that homed to head skin. Screening against homing to tail skin revealed that 12% of recovered $CX_3CX_3CX_3C$ (SEQ ID NO: 25) phage had the sequence CGATCEMQCPSGC (SEQ ID NO: 441).

Specificity for the skin homing peptides was also determined by competition experiments with GST-fusion peptides. Figure 3B shows that a GST-CVALCREACGEGC (SEQ ID NO: 3) fusion peptide coadministered with CVALCREACGEGC (SEQ ID NO: 3) inhibited homing to the skin, whereas GST had no effect on homing. The inhibitory effect of the GST-GFE-1 on homing was about 55% when injecting 500 µg of the GST-CVALCREACGEGC (SEQ ID NO: 3) fusion protein (Figure 3B).

These results demonstrate that *in vivo* panning can be used to screen phage display libraries in order to identify phage expressing peptides that home to skin and that such homing is specific.

C. Pancreas is the selected organ:

          After two or three rounds of *in vivo* panning
of mice injected with a cyclic CX₇C (SEQ ID NO: 24)
phage display library, various pancreas homing peptides
5   were identified (Table 3). In particular, the peptide
sequence SWCEPGWCR (SEQ ID NO: 4) appeared repeatedly
in the pancreas. To determine the specificity of
SWCEPGWCR (SEQ ID NO: 4), a phage displaying the
sequence was amplified individually, diluted to the
10 same input titer and administered to mice. Following
administration, control brain organ was removed and the
number of TU of phage in each pancreas and was
determined. The results shown in Figure 2, reveal that
10x more phage displaying the peptide sequence
15 SWCEPGWCR (SEQ ID NO: 4) bound to pancreas than to
brain and additional experiments revealed up to 20x
enrichment in pancreas as compared to brain (Table 1).
In addition, SWCEPGWCR (SEQ ID NO: 4) exhibited a 22x
enrichment of phage to the pancreas as compared to
20 unselected phage (see Figure 2). Thus, substantial
enrichment of phage binding to the pancreas was
observed in comparison to control tissue (brain) and to
unselected phage.

          These results demonstrate that *in vivo*
25 panning can be used to identify molecules that
selectively home to pancreas. In addition, the results
indicate that *in vivo* panning identifies independent
phage encoding the same peptide.

D. Retina is the selected tissue

Rats injected with a cyclic CX_7C (SEQ ID NO: 24) phage display library were subjected to *in vivo* panning and, after three rounds, the peptide sequences

5   CSCFRDVCC (SEQ ID NO: 5) and CRDVVSVIC (SEQ ID NO: 6) were identified in retina. Because of small tissue sample size, the phage isolated could not be accurately quantitated. Thus, the selectivity of phage displaying the peptides was determined by individually amplifying

10   the phage displaying the sequence and administering the phage to rats with a control phage fdAMPLAY88. This fd-ampicillin phage is similar to fd-tetracycline (fuse 5-based) in that it has the same infectivity.

Rats were injected with an equal amount of

15   the CSCFRDVCC (SEQ ID NO: 5) or CRDVVSVIC (SEQ ID NO: 6) and the fdAMPLAY88 phage. Following administration, homing to retina was evaluated by comparing the number of TU of the selected phage on tetracycline plates and fdAMPLAY88 on ampicillin plates

20   recovered from retina.

The results revealed that CSCFRDVCC (SEQ ID NO: 5) showed a 3x enrichment and CRDVVSVIC (SEQ ID NO: 6) showed a 2x enrichment in retina compared to control fdAMPLAY88 phage. Thus, substantial enrichment

25   of phage binding to the retina was observed in comparison to control phage.

Additional retina homing peptides were obtained and the amino acid sequences were determined for the inserts (Table 6, below). Peptides that

30   appeared more than one time are indicated. In

particular, the RDV tripeptide motif was present in
several different sequence contexts, indicating that
the nucleic acids encoding the peptides were derived
from a number of independent phage.

5   These results indicate that the selection of
the peptides containing the RDV motif represents the
selective binding of several independent phage
displaying peptides having the RDV sequence and is not
an artifact due, for example, to phage amplification.
10   In addition, in some cases, phage that expressed
peptides having the same amino acid sequence were
encoded by oligonucleotides having different sequences,
therefore confirming that homing of a particular phage
to retina is due to the specific peptide expressed on
15   the phage.

        These results further demonstrate that the *in
vivo* panning method is a generally applicable method
for screening a library to identify, for example, phage
expressing peptides that home to a selected organ or
20   tissue, including organs and tissues containing a
component of the RES. Database searches did not reveal
any significant homology of the pancreas, lung, skin or
retina homing peptides to known ligands for endothelial
cell receptors.

25   **EXAMPLE III**

          IMMUNOHISTOLOGIC ANALYSIS OF LUNG, PANCREAS
                  AND SKIN HOMING PEPTIDES

        This example demonstrates the localization of
lung, pancreas and skin homing molecules using
30   immunohistologic examination.

Phage displaying homing peptides were detected in lung, pancreas and skin by immunostaining histologic sections obtained 5 min after administration of phage expressing a lung, pancreas or skin homing

5   peptide ("peptide-phage") to a mouse. Following administration of the peptide-phage, mice were handled as described above and various organs, including lung, pancreas and skin, were removed and fixed in Bouin's solution (SIGMA). Histologic sections were prepared

10 and reacted with anti-M13 (phage) antibodies (Pharmacia Biotech; see U.S. Patent No. 5,622,699, *supra*, 1997; Pasqualini and Ruoslahti, *supra*, 1996). Visualization of the bound anti-M13 antibody was performed using a second antibody conjugated to peroxidase (SIGMA)

15 according to the manufacturer's instructions.

Phage displaying the lung homing peptide, GFE-1 (SEQ ID NO: 1), were administered intravenously to mice and, after 5 minutes of circulation, the lung was isolated and processed as described above.

20 Immunohistochemical staining of the alveolar capillaries was observed and no preference for any anatomical portion was detected. However, staining of bronchiolar walls and some larger blood vessels was absent. Mice injected with unselected phage did not

25 exhibit lung staining, and no staining was observed in pancreas and skin after injection of GFE-1 (SEQ ID NO: 1).

Similar experiments were performed in pancreas using phage displaying the pancreas homing

30 peptide, SWCEPGWCR (SEQ ID NO: 4). In these experiments, histological samples of the pancreas as

well as control organs and tissues including lung and
skin were prepared and examined by immunostaining as
described above. The results revealed staining in the
capillaries and larger blood vessels of the exocrine
5 pancreas whereas little if any staining of the
endocrine pancreas was detected. Again, unselected
phage did not stain pancreas, nor was any staining
observed in lung and skin of mice injected with phage
displaying SWCEPGWCR (SEQ ID NO: 4). Interestingly,
10 some staining of blood vessels within the uterus was
observed for the SWCEPGWCR (SEQ ID NO: 4) peptide.
Moreover, after intravenous injection of phage
displaying SWCEPGWCR (SEQ ID NO: 4), the phage was
recovered from uterus at a 6x higher level in
15 comparison to unselected phage. Thus, SWCEPGWCR (SEQ
ID NO: 4) homes to both pancreas and uterus.

Experiments were performed in skin using
phage displaying the skin homing peptide CVALCREACGEGC
(SEQ ID NO: 3). In these experiments, histological
20 samples from the skin as well as control organs and
tissues including lung and pancreas were prepared and
examined by immunostaining as described above. The
results revealed staining in blood vessels of the
hypodermis whereas little if any staining of the dermis
25 was detected. Again, unselected phage did not stain
these blood vessels, and no staining was observed in
control the lung and pancreas of mice injected with
phage displaying CVALCREACGEGC (SEQ ID NO: 3).

All phage, including unselected phage, caused
30 staining of the liver and spleen. This result is
consistent with the capture of phage by a component of
the RES which was previously described.

These results demonstrate that lung, pancreas and skin homing peptides selectively home to lung, pancreas and skin, particularly to the vasculature. In addition, these results reveal that organs and tissues

5 can exhibit differences of the staining patterns within particular regions, presumably reflecting the differential expression of a target molecule within the organ or tissue. Immunohistochemical analysis provides a convenient assay for identifying the localization and

10 distribution of phage expressing lung, pancreas and skin homing peptides.

EXAMPLE IV

THE RECEPTOR FOR THE GFE-1 LUNG HOMING PEPTIDE IS MEMBRANE DIPEPTIDASE

15 This example demonstrates that the receptor for "GFE" containing peptides such as GFE-1 (SEQ ID NO: 1) is membrane dipeptidase. This example further demonstrates that GFE-1 (SEQ ID NO: 1) binds and inhibits membrane dipeptidase activity.

20 A. GFE-1 phage bind selectively to lung primary cells

As described above, CGFECVRQCPERC (SEQ ID NO: 1; GFE-1) phage bind to mouse lung vasculature when injected *in vivo*. A phage binding assay on lung primary cells was performed to determine if the

25 specificity of CGFECVRQCPERC (SEQ ID NO: 1; GFE-1) phage binding to lung tissue *in vivo* could be reconstituted *in vitro*.

Phage binding to primary cells was performed as follows. Briefly, Balb/c mice (Harlan Sprague

Dawley) were anesthetized with 0.017 ml/g of Avertin as described in Gardner et al., _Lab. Animal Sci._ 45:199-204 (1995), which is incorporated herein by reference. Under deep anesthesia, mice were perfused

5   through the heart with 10 ml Dulbecco's modified Eagle's medium (DMEM; Irvine Scientific; Santa Ana, CA). The lungs, kidneys and brain were then collected, minced, placed in 2 ml of DMEM containing 0.5 % BSA (Intergen; Purchase, NY) and 0.5 µg/ml collagenase V

10   (SIGMA) and incubated at 37 °C for 25 minutes. Following collagenase treatment, the tissue was forced through a 70 µM pore cell strainer (Becton Dickinson; Franklin Lake, NJ). The filtered cells were washed once with 10 ml DMEM supplemented with 10% serum. The

15   phage particles used in the binding assay were amplified and purified, and the phage-displayed inserts were sequenced as described in Smith and Scott, _supra_, 1993. To ensure an equal input of the different phage to be tested, phage were titered several times using

20   K91Kan bacteria (Smith and Scott, _supra_, 1993).

For the binding reaction, 10^9 transducing units (TU) of phage were incubated with 5 X 10^6 cells in 1 ml DMEM supplemented with 10% serum. The binding was performed at 4 °C for 2 hours with gentle

25   agitation. After the binding reaction, the cells were washed four times with 1 ml of DMEM supplemented with 10% serum at room temperature for 5 to 10 minutes each time. The cells were centrifuged, and the cell pellet resuspended in 100 µl of DMEM and transferred to a new

30   tube. The phage bound to the cells were rescued by adding 1 ml of K91Kan bacterial culture (Smith and Scott, _supra_, 1993) followed by incubation at room temperature for 30 minutes. The bacteria were then

diluted in 10 ml of LB culture media supplemented with
0.2 µg/ml tetracycline, and incubated for another 30
minutes at room temperature. Serial dilutions of the
bacterial culture were plated on LB plates containing
5   40 µg/ml tetracycline. Plates were incubated at 37 °C
overnight before the colonies were counted (transducing
units, TU).

The results of the phage binding assay on
primary lung cells *in vitro* showed that primary lung
10 cells bound about 60-fold more CGFECVRQCPERC (SEQ ID
NO: 1; GFE-1) phage than insertless fd-tet phage.
Binding of CGFECVRQCPERC (SEQ ID NO: 1; GFE-1) phage to
kidney cells was also higher than fd-tet binding
(t test, p<0.02) although this binding was much lower
15 than the GFE-1 (SEQ ID NO: 1) binding on lung cells.
In *in vivo* studies, no specific phage homing to kidney
was detected when the GFE-1 (SEQ ID NO: 1) phage was
injected intravenously. The GFE-1 (SEQ ID NO: 1) phage
showed no specific binding to primary brain cells *in*
20 *vitro*.

GFE-1 (SEQ ID NO: 1) phage binding to lung
cells was inhibited by almost 70% in the presence of
150 µM GFE-1 (SEQ ID NO: 1) peptide, whereas the same
concentration of a control peptide, GRGESP (SEQ ID NO:
25 442), had no effect. The non-specific binding of the
insertless fd-tet phage was not affected by the
presence of GFE-1 (SEQ ID NO: 1) or control peptides.
These results demonstrate that the selective *in vivo*
binding of the GFE-1 (SEQ ID NO: 1) peptide sequence to
30 lung endothelium can be reconstituted in an *in vitro*
assay on total lung primary cells and that whole lung

cell extracts contain sufficient amounts of GFE-1
receptor for isolation.

B. The GFE-1 (SEQ ID NO: 1) peptide binds a 55 kDa
5 surface protein

        To detect only the cell surface molecules
that bind to GFE-1 (SEQ ID NO: 1), mouse lung
endothelial surface proteins were biotinylated *in vivo*,
and a total lung extract prepared. The labeled extract
10 was first fractionated on a GFE-1 (SEQ ID NO: 1)
peptide affinity column; subsequently, the column was
washed, and bound proteins were eluted with a GFE-1
(SEQ ID NO: 1) peptide solution.

        In vivo biotinylation of endothelial cell
15 surface proteins was performed as previously described
in De La Fuente et al., Am. J. Physiol. 272: L461-L470
-(1997), which is incorporated herein by reference, with
several modifications. Briefly, Balb/c mice were
anesthetized with Avertin and perfused slowly through
20 the heart for 10 to 15 minutes with approximately 15 ml
of PBS containing 0.5 mg/ml of sulfo-NHS-LC-biotin
(Pierce; Rockford, IL). Well perfused lungs or control
tissues such as brain were then collected and incubated
on ice for 20 minutes. The tissues were then
25 homogenized for preparation of extracts as described
further below.

        For preparation of extracts, mouse or rat
lungs were first minced and then homogenized with a
Brinkman homogenizer (Brinkman; Wesbury, NY) in a
30 minimal volume (2.5 ml/g of tissue) of cold PBS
containing 100 mM N-octyl-β-D-glucopyranoside

(Calbiochem; La Jolla, CA) with 1 mM
phenylmethylsulfonyl fluoride (PMSF), 20 μg/ml
aprotinin and 1 μg/ml leupeptin (PBS/octylglucoside).
The homogenized tissue was incubated on ice for 2
5 hours, and then centrifuged at 12,000 x g for 30
minutes to remove cell debris. The pooled supernatants
were cleared of any debris prior to affinity
chromatogaphy.

GFE-1 (SEQ ID NO: 1) affinity chromatography
10 was performed according to the general principles
established by Pytela et al. for the isolation of
integrins by RGD peptide chromatography (Pytela et al.,
Cell 40:191-198 (1985); Pytela et al., Methods Enzym.
144:475-489 (1987), each of which is incorporated
15 herein by reference). All steps were performed at 4
°C. Briefly, GFE-1 (SEQ ID NO: 1) or control peptides
(AnaSpec; San Jose, CA) were coupled to CNBr-activated
Sepharose 4B according to the manufacturer's
instructions (Pharmacia Biotech; Uppsala, Sweden). The
20 matrix contained approximately 2 mg/ml of peptide. The
biotin-labeled extract from 2 mouse lungs was applied
to 500 μl of the affinity matrix equilibrated in column
buffer (PBS containing 50 mM octylglucoside and 1 mM
PMSF). The extract was applied to the column, and the
25 flow-through re-applied over a period of 90 minutes.
The column was then washed with 20 volumes of column
buffer. Elution with the synthetic GFE-1 (SEQ ID
NO: 1) peptide was carried out by slowly washing the
column over a period of 1 hour with 2 volumes of column
30 buffer supplemented with 1 mg/ml of GFE-1 peptide (SEQ
ID NO: 1) . The remaining proteins bound to the column
were eluted with 8 M urea.

Eluates were concentrated 5-fold using a
Centricon 10,000 MWCO column (Amicon; Beverly, MA).
Aliquots of each elution were then separated by
SDS-PAGE using pre-cast polyacrylamide 4-12% gels
5   (Novex; San Diego, CA). For the experiments done with
biotin-labeled extracts, the proteins were transferred
to a PVDF membrane (Millipore; Bedford, MA), blotted
with streptavidin-HRP (Pierce; Rockford, IL) and
developed with the ECL chemiluminescence system (NEN;
10   Boston, MA).

As shown in Figure 4 (left panel), a 55 kDa
biotinylated protein was selectively eluted by GFE-1
(SEQ ID NO: 1). Prior to elution, the washes from the
column showed no detectable biotinylated proteins;
15   subsequent addition of 8 M urea eluted many
biotinylated proteins that were retained non-
specifically in the column. No proteins in the 55 kDa
range were detected after performing the same procedure
on a control peptide (GRGESP; SEQ ID NO: 442) column
20   (Figure 4; right panel). As an additional control, an
in vivo biotinylated brain cell extract was
fractionated through a GFE-1 (SEQ ID NO: 1) peptide
column under the same conditions; no biotinylated
proteins from the brain extract specifically bound to
25   the GFE-1 (SEQ ID NO: 1) peptide column (data not
shown).

These results indicate that GFE-1 peptide
(SEQ ID NO: 1) specifically binds to a 55 kDa lung
vascular surface protein. Under non-reducing
30   conditions, the 55 kDa protein migrated as a 110 kDa
band (data not shown). Thus, these results further

indicate that the GFE receptor is a disulfide-linked homodimer.

C. The 55 kDa GFE-1 (SEQ ID NO: 1) receptor is membrane dipeptidase

5   GFE-1 (SEQ ID NO: 1) phage can selectively target rat lung blood vessels when injected in the circulation. To purify a larger amount of the 55 kDa protein than could be obtained from mouse tissues, a non-biotinylated extract was prepared from 100 frozen
10   rat lungs (Pel-Freez Biologicals; Rogers, AR). The large scale extract was prepared essentially as described above with the addition of a second extraction of the pellet with a minimal volume of PBS/octylglucoside.

15   The large scale extract was fractionated on a GFE-1 (SEQ ID NO: 1) peptide affinity column as described above using 3 ml of affinity matrix. A 55 kDa protein that was detectable by Coomassie blue staining was eluted from the column by GFE-1 peptide
20   SEQ ID NO: 1 (data not shown). This protein, which co-migrated with the 55 kDa surface protein isolated from mouse lung, was subjected to tryptic digestion and sequenced by mass spectrometry at the Harvard University Microchemistry Facility (Boston, MA) by
25   microcapillary reverse phase HPLC tandem mass spectrometry (µLC/MS/MS) on a Finnigan LCQ quadrupole ion trap mass spectrometer.

      Two tryptic peptides derived from the 55 kDa protein, YPDLIAELLR (SEQ ID NO: 444) and
30   TTPVIDGHNDLPWQMLTLFNNQLR (SEQ ID NO: 445), showed

complete identity with rat membrane dipeptidase
(EC 3.4.13.19), also known as microsomal dipeptidase,
dehydropeptidase-1 or MDP. Several other peptides
sequences indicated the presence of rat IgG in the
5 sample. Contamination of the sample with IgG is
expected in this molecular weight range, given the
abundance of IgG in an extract from unperfused lungs.

        To confirm that membrane dipeptidase (MDP) is
the GFE-1 (SEQ ID NO: 1) peptide binding protein, the
10 55 kDa protein was assayed for membrane dipeptidase
activity. Samples from the affinity chromatography
wash fraction and the GFE-1 (SEQ ID NO: 1) peptide
eluate (Figure 4) were incubated in the presence of the
specific MDP substrate Gly-D-Phe, and D-Phe detected
15 fluorimetrically exactly as described in Heywood and
Hooper, *supra,* 1995 (see, also, Keynan et al., *supra,*
1996). Briefly, the samples were first incubated at 37
°C for 3 hours with the MDP substrate Gly-D-Phe (1 mM;
SIGMA). The released D-Phe was then converted to 6,6'-
20 dihydroxy-[1,1'-biphenyl]-3,3'-diacetic acid in the
presence of D-amino acid oxydase (Type I; SIGMA) and
peroxidase (Type VI; SIGMA; Heywood and Hooper, *supra,*
 1995). Fluorescence was measured using an *fmax*
fluorescence microplate reader from Molecular Devices
25 (Sunnyvale, CA).

        Figure 5 shows a time course of the
conversion of D-Phe into a fluorescent compound in
samples from the affinity chromatography wash fraction
and GFE-1 peptide eluate described above (Figure 4).
30 While the wash fraction showed only a baseline level of
fluorescence, the GFE-1 (SEQ ID NO: 1) peptide eluate
contained high membrane dipeptidase activity as

illustrated by the time-dependent conversion of D-Phe
(see Figure 5). In addition, GFE-1 (SEQ ID NO: 1)
peptide eluate isolated from the rat lung extract also
showed strong MDP activity (data not shown). MDP
5 activity also was detected in the total lung extract,
although the specific activity was about 600-fold
higher for the GFE-1 peptide (SEQ ID NO: 1) eluate (200
nmol D-Phe/min/mg) than for the total rat lung extract
(0.34 nmol D-Phe/min/mg).

10 D. GFE-1 (SEQ ID NO: 1) and GFE-2 (SEQ ID NO: 2) phage
bind to cells transfected with MDP

The COS-1 cell line, which is known to have
low or no detectable level of MDP activity, has been
used extensively to study MDP structure and function
15 (Keynan et al., *supra,* 1996; Keynan et al., Biochem. J.
326:47-51 (1997)). COS-1 cells were trasfected with
murine MDP and used to assay binding to SEQ ID NOS: 1
and 2 as described below.

For transfection into COS-1 cells, a murine
20 MDP expression vector was prepared as follows. Total
mouse lung RNA was isolated using Qiagen RNA
purification columns (Qiagen; Santa Clarita, CA) and
used as a template for first strand cDNA synthesis with
reverse transcriptase and a mixture of random hexamers
25 and poly-dT oligonucleotides (GIBCO/BRL; Grand Island,
NY). Mouse MDP cDNA (Pasqualini et al., J. Cell Biol.
130:1189-1196 (1995)) was amplified from the cDNA pool
by PCR using the oligonucleotide pair:
CCGCTGGTACCGCAGATCCCTGGGGACCTTG (SEQ ID NO: 446), which
30 contains a Kpn I adaptor, and
TCTTTCTAGAGCTCAGAGAGCACTGGAGGAG (SEQ ID NO: 447), which

contains an Xba I adaptor, using Taq polymerase from GIBCO/BRL. The amplified 1.3 kb murine MDP cDNA was digested with Kpn I and Xba I, and inserted into the same sites of the pcDNA3 expression vector (Invitrogen; Carlsbad, CA) using DNA restriction enzymes and T4 DNA ligase from New England Biolabs (Beverly, MA). Successful cloning of the murine MDP cDNA was confirmed by DNA sequencing. Transfection of the COS-1 cells was performed with the Superfect Reagent from Qiagen as recommended by the manufacturer.

Phage binding to COS-1 cells transfected with MDP was determined as follows. Briefly, 10^7 COS-1 cells were transfected with 10 µg of either the MDP expression vector or the vector alone. After 48 hours, cells were scraped gently from the dish, washed once, and subjected to the phage binding assay or membrane dipeptidase activity assay described above. For the phage binding assay, 5 x 10^6 cells and 10^{10} transducing units (TU) of phage input were used. For measurement of membrane dipeptidase activity, 10^6 cells were lysed in 100 µl of PBS/octylglycoside without protease inhibitors. A 10 µl aliquot of the extract was used to measure MDP activity.

As shown in Figure 6A, COS-1 cells transfected with murine MDP showed at least 15-fold higher MDP activity than mock transfected cells. Furthermore, GFE-1 (SEQ ID NO: 1) phage bound COS-1 cells transfected with MDP. As shown in Figure 6B, the GFE-1 (SEQ ID NO: 1) phage bound 4-fold more to MDP transfected cells than to mock transfected cells. Negative control phage, the fd-tet phage and a skin-homing phage (CVALCREACGEGC; SEQ ID NO: 3)

displaying a peptide with structural features similar
to those of the GFE-1 (SEQ ID NO: 1) peptide, showed no
specific binding to cells expressing MDP as compared to
mock transfected cells. In addition, Figure 6B shows

5   that GFE-2 (SEQ ID NO: 2) phage also bound MDP
transfected cells; the binding was weaker than the
GFE-1 (SEQ ID NO: 1) binding in agreement with the *in
vivo* lung homing data described above. The binding of
both GFE-1 (SEQ ID NO: 1) and GFE-2 (SEQ ID NO: 2)

10   phage to MDP-transfected cells was completely inhibited
by GFE-1 peptide (SEQ ID NO: 1; data not shown). These
results indicate that the GFE-1 (SEQ ID NO: 1) and
GFE-2 (SEQ ID NO: 2) peptides bind the same receptor.

E. GFE-1 (SEQ ID NO: 1) can inhibit MDP activity

15

        The metabolism of the tripeptide glutathione
involves cleavage of the tripeptide by γ-glutamyl
transpeptidase to form glutamate and cysteinylglycine
(Cys-Gly). The dipeptide Cys-Gly is subsequently

20   recognized and cleaved by MDP, which cleaves only
dipeptides. The amino acid sequence of glutathione is
similar to the N-terminal portion of the GFE-1 peptide
CGFECVRQCPERC (SEQ ID NO: 1), with the first two first
amino acids of GFE-1 being Cys-Gly.

25   GFE-1 (SEQ ID NO: 1) was assayed for the
ability to inhibit hydrolysis of Gly-D-Phe by MDP.
Fluorimetric detection of D-Phe was performed as
described above. Figure 7 shows that GFE-1 (SEQ ID
NO: 1) inhibited hydrolysis of the Gly-D-Phe substrate

30   (0.5 mM) in a dose-dependent manner. A control cyclic
peptide (CARAC; SEQ ID NO: 443) did not inhibit the
enzyme.

These results indicate that GFE-1 (SEQ ID NO: 1) can act as a competitive inhibitor of MDP activity.

EXAMPLE V

5   **GFE-1 (SEQ ID NO: 1) INHIBITS LUNG METASTASIS**

This example demonstrates that the GFE-1 peptide SEQ ID NO: 1 can inhibit experimental lung metastasis in mice.

Experimental lung metastasis was induced in
10   mice using C8161 human melanoma cells essentially as described in Arap et al., Science 279:377-380 (1998) and Pasqualini et al., Nature Med. 11:1197-1203, each of which is incorporated herein by reference. Briefly, C8161 cells were cultured to 75% confluence and then
15   collected with 2.5 mM EDTA/PBS. The C8161 cancer cells were injected into the tail vein of female nude BALB/c mice (two months old) at a concentration of 10^5 cells per animal. Two sets of five mice were injected with the cells alone; cells with 250 µg control CARAC
20   peptide (SEQ ID NO: 443) or cells with 250 µg GFE-1 peptide (SEQ ID NO: 1). Each injection was in a total volume of 200 µl.

Aliquots of the melanoma cells and peptide mixture were cultured overnight to confirm that the
25   peptides do not affect viability of the tumor cells. Neither the GFE-1 (SEQ ID NO: 1) or CARAC (SEQ ID NO: 443) peptides exhibited toxicity to the melanoma cells (data not shown).

Mice were sacrificed five weeks after injection, and the lungs collected and weighed. As shown in Figure 8, the lung weight of mice administered GFE-1 (SEQ ID NO: 1) peptide was signficantly less than the lung weight seen in mice administered melanoma cells alone ("vehicle") or melanoma cells with control peptide CARAC SEQ ID NO: 443 ("control peptide"). These results indicate that GFE-1 can inhibit lung metastasis of human cancer cells.

A similar lung metastasis experiment was performed using a GFE-1 (SEQ ID NO: 1) glutathione S-transferase fusion protein prepared as described in Rajotte et al., *J. Clinical Invest.* 102:430-437 (1998), which is incorporated herein by reference. The GFE-1 (SEQ ID NO: 1) glutathione S-transferase fusion protein inhibited the increase in lung weight resulting from injection of C8161 human melanoma cells in a manner similar to the results observed with peptide SEQ ID NO: 1 as shown in Figure 8.

These results indicate that GFE-1 (SEQ ID NO: 1) can inhibit lung metastasis and that MDP serves as a receptor for metastasizing tumor cells on lung vasculature.

EXAMPLE VI

PREPARATION OF Z-2-ACYLAMINO-3-MONOSUBSTITUTED PROPENOATE MDP-BINDING HOMING MOLECULES

This example demonstrates several methods for preparation of MDP-binding molecules having Structure 1.

A. Method for preparation of an MDP-binding homing
molecule having Structure 1

        An MDP-binding homing molecule having
Structure 1 is made by condensing directly the
5   appropriate 2-keto acid and amide as follows:

$$R^3CH_2\overset{\overset{\displaystyle O}{\|}}{C}CO_2H$$

$$+$$

$$R^2\overset{\overset{\displaystyle O}{\|}}{C}NH_2$$
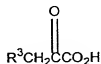
wherein R^2 and R^3 are as defined. The general reaction
10 conditions involve mixing approximately 14: 1 parts of
the acid to the amide in an inert solvent such as
toluene or methyl isovalerate and heating at reflux
with azeotropic removal of water for 3-48 hours,
preferably 5-24 hours. The solution when cooled
15 normally yields the product in crystalline form. If
desired, the product is isolated using a base
extraction process. The product is recrystallized
using generally known techniques.

        An optional modification of this procedure
20 requires an additional small amount of p-
toluenesulfonic
acid as catalyst during the reaction.

B. Method for preparation of an MDP-binding homing molecule having Structure 1

An MDP-binding homing molecule having Structure 1 is prepared using an α-amino acid, t-butyl
5 ester in reaction with an acid chloride as follows:

$$R^2 \!-\!\!-\!\!-\!\!\overset{\displaystyle O}{\underset{\displaystyle \|}{C}}Cl$$

+

$$R^3 \!-\!\! CH_2 \!-\!\! \overset{\displaystyle H}{\underset{\displaystyle NH_2}{C}} \!-\! COO \!-\! C(CH_3)_3$$
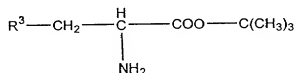
The reaction takes place in the presence of base, such
10 as triethylamine, in a solvent such as methylene
chloride. The resulting N-acylated product is then
oxidized by treatment with t-butyl hypochlorite
followed by addition of sodium methoxide. This yields
the 2-methoxy derivative or its elimination product,
15 the α,β-unsaturated ester. Further treatment with
anhydrous hydrochloric acid converts either the
2-methoxy derivative or the unsaturated ester (or the
mixture of both) to the desired α,β-unsaturated free
acid.

$$R^3CH_2CHCO_2C(CH_3)_3$$

$$NHCR^2$$

$$O$$

$$OCH_3$$

$$R^3CH_2CCO_2C(CH_3)_3$$

$$NHCR^2$$

$$O$$

$$R^3CH = CCO_2C(CH_3)_3$$

$$NHCR^2$$

$$O$$
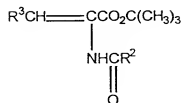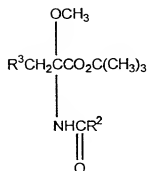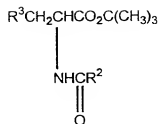
Some compounds in which R^3 has a terminal substituent
5   which is an amino, quaternary nitrogen, thiol or
carboxyl, derivative can be made most conveniently
from an intermediate having a terminal bromine. In
this case the intermediate has the structure

$$\underset{(CH_2)_n}{\overset{H}{\diagdown}} \underset{\underset{H}{|}}{N} - \underset{\underset{O}{\|}}{C} - R^2 \quad COOR^1$$
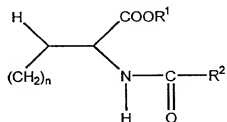
10   where n is the number of carbons in the desired
hydrocarbon chain (e.g., from 3-7).

In order to prepare R^3 having a terminal trimethylammonium substituent, the bromo intermediate is reacted with trimethylamine; to yield the amino, the bromo intermediate is reacted with ammonia; the

5 guanidino reaction is with guanidine; to prepare the thio derivatives, including 2-amino-2-carboxyethylthio, the bromo compound is reacted with cysteine HCl, or the appropriate mercaptan. Derivatized amino, such as formamidino, ureido, and acylamide (acetamido) are

10 made from the compounds having an amino group by reacting with o-benzyl formimidate HCl, potassium cyanate and the appropriate acyl anhydride (acetic anhydride) respectively.

C. Method for preparation of an MDP-binding homing

15 molecule having Structure 1

Another route for preparing compounds when R^3 is a terminally substituted thio derivative utilizes a chloroketo ester intermediate as follows:

$$Cl{-}(CH_2)_n{-}\overset{\overset{\textstyle O}{\|}}{C}{-}CO_2R$$
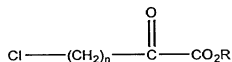
20 in reaction with the desired amide,

$$R^2{-}\overset{\overset{\textstyle O}{\|}}{C}{-}NH_2$$
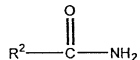
in toluene at reflux in the presence of a catalytic amount of p-toluenesulfonic acid. The resulting

intermediate is hydrolyzed to the acid; the chloro
group is then displaced in reaction with the
appropriate mercaptan. This reaction is valuable since
it permits use of the chiral amide, thereby preparing a
5 functionalized side chain. Alternatively, the mixture
of Z+E isomers prepared after the mercaptan
condensation is directly isomerized into the Z form by
adding acid to a pH of about 3, and heating to about 90
°C for 30 minutes. Only the Z form remains, and
10 recovery is simple and straight-forward.

D. Preparation of Sodium Z-7-(L-amino-2-
carboxyethylthio)-2- (2,2-dimethylcyclopropane
carboxamido)-2-heptenoic acid

1. Grignard preparation of ethyl-7-chloro-2-
15 oxoheptanoate

Equimolar amounts (8 moles each) of I-bromo-
5-chloropentane and magnesium are reacted in
tetrahydrofuran (THF) (960 ml) at 25 °C. The flask is
charged with the magnesium in the THF, and the
20 bromochloropentane added over 1 hour, then aged 2
hours. After the reaction is judged complete, the
reaction solution is added (cooled -15 °C, to 16 moles
of diethyloxalate in 1856 ml THF, while maintaining the
temperature at 10 degrees C. 3N HCl is added to
25 quench, keeping the temperature below 25 °C. After
stripping solvents, the calculated yield is 48.8% of
the ethyl-1-chloro-6-oxoheptenoate.

2. Condensation and Hydrolysis

S-2,2-dimethylcyopropyl carboxamide (1017 g),

2143.6 g of ethyl-7-chloro-2-ketoheptanoate, 9 liters
of toluene and 12 g of p-toluene sulfonic acid are
charged to a 22 L flask, and heated to reflux with
stirring. After 23 hours, liquid chromatography shows
5   the expected product ratio, and 4 L of toluene are
removed under slightly reduced pressure. The pot is
charged with water, neutralized to pH 7 with 2 N
NaOH, and vacuum distilled leaving a final pot volume
of about 5 liters. This is hydrolyzed by adding 1760 g
10 of 50% aqueous NaOH (4 liters water) and stirring
overnight. The flask is charged with 4 L methylene
chloride, and pH adjusted to 8.8 using HCl. Unreacted
amide crystallizes out. The organic layers are
separated from water, and then evaporated. The gummy
15 residue is dissolved in 8 L water containing 720 g, 50%
NaOH, and to this solution is charged 1818 g L-cysteine
HCl, H_2O, 2 kg ice, 2484 g 50% NaOH and 1 L water. The
pH of this solution, after aging overnight at room
temperature, is adjusted to 3.0 with concentrated HCl,
20 and the resulting gummy suspension is heated to 95 °C
to afford a clear solution. After 30 minutes, no E
isomer is detected by 1c. After work-up and
purification, the overall yield is 2060 g, 87% yield.
This material is recrystallized from acetonitrile.
25 1500 g of the recrystallized material is dissolved in 6
L water and 910 ml 3.88 N NaOH, then neutralized to pH
7, and lyophilized to afford 1569 g (98.6%) of the
title compound. Analysis: Calcd,: C, 50.52; H, 6.62;
N, 7.36; S, 8.43; Na, 6.04. Found: C, 50.71; H, 6.78;
30 N, 7.49; S, 8.52; Na, 5.92

        All journal article, reference, and patent
citations provided above, in parentheses or otherwise,

whether previously stated or not, are incorporated
herein by reference.

5   Although the invention has been described
with reference to the examples provided above, it
should be understood that various modifications can be
made without departing from the spirit of the
invention. Accordingly, the invention is limited only
by the claims.

TABLE 2

PEPTIDES FROM PHAGE RECOVERED FROM LUNG

CIKGNVNC	(32)	CRHESSSC	(33)
CLYIDRRC	(34)	CYSLGADC	(35)
CSKLMMTC	(349)	CGFELETC*	(2)
CNSDVDLC	(36)	CVGNLSMC*	(37)
CEKKLLYC	(38)	CKGQRDFC*	(39)
CTFRNASC	(40)	CNMGLTRC*	(41)
CHEGYLTC*	(42)	CGTFGARC	(43)
CIGEVEVC*	(16)	CRISAHPC	(44)
CLRPYLNC*	(45)	CSYPKILC	(46)
CMELSKQC*	(47)	CSEPSGTC	(48)
CGNETLRC	(49)	CTLSNRFC	(50)
CMGSEYWC	(51)	CLFSDENC*	(52)
CAHQHIQC	(53)	CKGQGDWC	(54)
CAQNMLCC	(55)	CWRGDRKIC*	(56)
CLAKENVVC*	(13)	CIFREANVC	(57)
CRTHGYQGC	(58)	CERVVGSSC	(59)
CKTNHMESC	(60)	CYEEKSQSC	(61)
CKDSAMTIC	(62)	CTRSTNTGC	(63)
CMSWDAVSC*	(64)	CKWSRLHSC*	(65)
CMSPQRSDC	(66)	CLHSPRSKC	(67)
CPQDIRRNC	(68)	CLYTKEQRC	(69)
CQTRNFAQC	(70)	CTGHLSTDC	(71)
CQDLNIMQC	(72)	TRRTNNPLT	(73)
CGYIDPNRISQC	(74)	CTVNEAYKTRMC*	(75)
CRLRSYGTLSLC*	(76)	CAGTCATGCNGVC	(77)
CADYDLALGLMC	(78)	CPKARPAPQYKC	(79)
CSSHQGGFQHGC	(80)	CQETRTEGRKKC	(81)
CRPWHNQAHTEC*	(82)	CSFGTHDTEPHC	(83)

TABLE 2 (cont.)

CSEAASRMIGVC* (84)	CWEEHPSIKWWC* (85)	
CWDADQIFGIKC (86)	CVDSQSMKGLVC (87)	
CRLQTMGQGQSC (88)	CRPAQRDAGTSC (89)	
5 CGGRDRGTYGPC (90)	CGEVASNERIQC (91)	
CNSKSSAELEKC (92)	CVLNFKNQARDC (93)	
CRGKPLANFEDC (94)	CEGHSMRGYGLC (95)	
CRDRGDRMKSLC (96)	CDNTCTYGVDDC (97)	
CSAHSQEMNVNC (98)	CGAACGVGCRGRC (99)	
10 CGFECVRQCPERC* (1)	CLVGCRLSCGGEC (100)	
CRSGCVEGCGGRC (101)	CIARCGGACGRHC (102)	
CGGECGWECEVSC (103)	CGVGCPGLCGGAC* (104)	
CKWLCLLLCAVAC (105)	CSEGCGPVCWPEC (106)	
CGAACGVGCGGRC (107)	CSGSCRRGCGIDC (108)	
15 CGASCALGCRAYC (109)	CDTSCENNCQGPC (110)	
CSRQCRGACGQPC (111)	CYWWCDGVCALQC (112)	
CAGGCAVRCGGTC (113)	CGGACGGVCTGGC* (114)	
CGRPCVGECRMGC (115)	CLVGCEVGCSPAC (116)	
CPRTCGAACASPC (117)	CRGDCGIGCRRLC (118)	
20 CCFTNFDCYLGC (435)		

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 3

PEPTIDES FROM PHAGE RECOVERED FROM PANCREAS

EICQLGSCT	(119)	WRCEGFNCQ	(120)
RKCLRPDCG	(121)	SWCEPGWCR*	(4)
LACFVTGCL	(122)	GLCNGATCM*	(123)
DMCWLIGCG	(124)	SGCRTMVCV	(125)
QRCPRSFCL	(126)	LSCAPVICG	(127)
RECTNEICY	(128)	NECLMISCR	(129)
SCVFCDWLS	(130)	WACEELSCF	(131)
QNCPVTRCV	(132)	CATLTNDEC	(133)
CDNREMSC	(134)	CFMDHSNC	(135)
CGEYGREC	(136)	CHMKRDRTC	(137)
CKKRLLNVC	(138)	CLDYHPKC	(139)
CMTGRVTC	(140)	CNKIVRRC	(141)
CPDLLVAC	(142)	CSDTQSIGC	(143)
CSKAYDLAC	(144)	CSKKGPSYC	(145)
CTLKHTAMC	(146)	CTQHIANC	(147)
CTTEIDYC	(148)	CVGRSGELC	(149)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 4

PEPTIDES FROM PHAGE RECOVERED FROM GUT

YAGFFLV*	(150)	RSGARSS	(151)
CVESTVA	(152)	SRRQPLS*	(153)
SKVWLLL	(154)	QVRRVPE	(155)
YSGKWGW*	(156)	MVQSVG	(157)
LRAVGRA	(158)	MSPQLAT*	(159)
GAVLPGE	(160)	WIEEAER*	(161)
LVSEQLR	(162)	RGDRPPY	(163)
VRRGSPQ	(164)	RVRGPER	(165)
GISAVLS*	(166)	GGRGSWE	(167)
GVSASDW	(168)	FRVRGSP	(169)
SRLSGGT	(170)	WELVARS	(171)
MRRDEQR	(172)	GCRCWA	(173)
LSPPYMW	(7)	LCTAMTE	(18)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 5

PEPTIDES FROM PHAGE RECOVERED FROM SKIN

CYADCEGTCGMVC (174)	CWNICPGGCRALC* (175)
GPGCEEECQPAC (176)	CKGTCVLGCSEEC* (177)
CSTLCGLRCMGTC (178)	CMPRCGVNCKWAC (179)
CVGACDLKCTGGC (180)	CVALCREACGEGC* (3)
CSSGCSKNCLEMC* (181)	CGRPCRGGCAASC (182)
CQGGCGVSCPIFC (183)	CAVRCDGSCVPEC* (184)
CGFGCSGSCQMQC (185)	CRVVCADGCRFIC (186)
CTMGCTAGCAFAC (187)	CEGKCGLTCECTC (188)
CNQGCSGSCDVMC (189)	CASGCSESCYVGC (190)
CGGGCQWGCAGEC* (191)	CSVRCKSVCIGLC (192)
CPSNCVALCTSGC (193)	CVEGCSSGCGPGC (194)
CRVVCADGCRLIC (195)	CSTLCGLRCMGTC (196)
CFTFCEYHCQLTC (197)	CVDVCCDGCPVCC (437)
RVPLSGDVEH (438)	LRVMSFTSGQ (439)
RFSVGSLFGS (440)	CGATCEMQCPSGC (441)

Parentheses contain SEQ ID NO:.
* indicates sequences isolated more than once.

TABLE 6

PEPTIDES FROM PHAGE RECOVERED FROM RETINA

CRRIWYAVC (198)	CSAYTTSPC (199)
CSCFRDVCC* (5)	CTDKSWPC (200)
CTDNRVGS (201)	CTIADFPC (202)
CTSDISWWDYKC (203)	CTVDNELC (204)
CVGDCIGSCWMFC (205)	CVKFTYDC[2] (206)
CVSGHLNC (207)	CYGESQQMC (208)
CYTGETWTC (209)	CAVSIPRC (210)
CDCRGDCFC (211)	CDSLCGGACAARC (212)
CERSQSKGVHHC (213)	CFKSTLLC (214)
CFWHNRAC (215)	CGDVCPSECPGWC (216)
CGEFKVGC* (14)	CGLDCLGDCSGAC (217)
CGPGYQAQCSLRC (218)	CGSHCGQLCKSLC (219)
CHMGCVSPCAYVC (220)	CILSYDNPC (221)
CISRPYFC (222)	CKERLEYTRGVC (223)
CKERPSNGLSAC (224)	CKPFRTEC (225)
CKSGCGVACRHMC (226)	CLKPGGQEC (227)
CMDSQSSC* (228)	CMNILSGC (229)
CNIPVTTPIFGC (230)	CNQRTNRESGNC* (231)
CNRKNSNEQRAC (232)	CNRMEMPC (233)
CQIRPIDKC (234)	CAIDIGGAC (235)
CGRFDTAPQRGC (236)	CKRANRLSC (237)
CLLNYTYC* (238)	CLNGLVSMC (239)
CMSLGNNC (240)	CNRNRMTPC (241)
CQASASDHC* (242)	CQLINSSPC (243)
CQRVNSVENASC (244)	CRKEHYPC (245)
CRRHMERC (246)	CSGRPFKYC (247)
CTHLVTLC (248)	CTSSPAYNC (249)
CVTSNLRVC* (250)	CWDSGSHIC (251)
CERSHGRLC[1] (252)	CGNLLTRRC (253)
CINCLSQC (254)	CLRHDFYVC (255)

TABLE 6 (cont.)

CNSRSENC (256)	CRYKGPSC (257)	
CSHHDTNC (258)	CSRWYTTC (259)	
CYAGSPLC (260)	CQTTSWNC* (261)	
5 CQWSMNVC (262)	CRARIRAEDISC* (263)	
CRDVVSVIC (6)	CRREYSAC (264)	

Blast-Search:
[1]rat retinal guanylcyclase precursor EC4.6.1.2
10 [2]rat glutamate receptor subunit epsilon 1 precursor
No stainings for any motif tested, only evidence for
preferential homing are the RDV-containig phages in
comparison to an ampicillin-phage.

Parentheses contain SEQ ID NO:.
15 * indicates sequences isolated more than once.

TABLE 7

PEPTIDES FROM PHAGE RECOVERED FROM PROSTATE

EVQSAKW	(265)	KRVYVLG	(266)
GRLSVQV	(267)	WKPASLS	(268)
FAVRVVG	(269)	LVRPLEG	(270)
GFYRMLG	(271)	EGRPMVY	(272)
GSRSLGA	(273)	RVWQGDV	(274)
GDELLA	(275)	FVWLVGS	(276)
GSEPMFR	(277)	VSFLEYR	(22)
WHQPL	(278)	SMSIARL*	(21)
RGRWLAL*	(279)	QVEEFPC	(280)
LWLSGNW	(281)	GPMLSVM	(282)
WTFLERL	(283)	VLPGGQW	(284)
REVKES	(285)	RTPAAVM	(286)
GEWLGEC	(287)	PNPLMPL	(288)
SLWYLGA	(289)	YVGGWEL	(290)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 8

PEPTIDES FROM PHAGE RECOVERED FROM OVARY

EVRSRLS*	(10)	RVGLVAR*	(11)
AVKDYFR	(291)	GVRTSIW	(292)
RPVGMRK	(293)	RVRLVNL	(294)
FFAAVRS	(295)	KLVNSSW	(296)
LCERVWR	(297)	FGSQAFV	(298)
WLERPEY	(299)	GGDVMWR	(300)
VRARLMS	(301)	TLRESGP	(302)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 9

PEPTIDES FROM PHAGE RECOVERED FROM LYMPH NODE

WGCKLRFCS	(303	MECIKYSCL (304)
GICATVKCS	(305)	PRCQLWACT (306)
TTCMSQLCL	(307)	SHCPMASLC (308)
GCVRRLLCN	(309)	TSCRLFSCA (310)
KYCTPVECL	(311)	RGCNGSRCS (312)
MCPQRNCL	(313)	PECEGVSCI (314)
AGCSVTVCG*	(315)	IPCYWESCR (316)
GSCSMFPCS*	(317)	QDCVKRPCV (318)
SECAYRACS*	(319)	WSCARPLCG* (320)
SLCGSDGCR	(321)	RLCPSSPCT (322)
MRCGFSGCT	(323)	RYCYPDGCL (324)
STCGNWTCR	(325)	LPCTGASCP (326)
CSCTGQLCR	(327)	LECRRWRCD (328)
GLCQIDECR*	(329)	TACKVAACH (330)
DRCLDIWCL*	(331)	XXXQGSPCL (332)
PLCMATRCA*	(333)	RDCSHRSCE* (334)
NPCLRAACI*	(335)	PTCAYGWCA* (336)
LECVANLCT*	(337)	RKCGEEVCT* (338)
EPCTWNACL*	(339)	LVCPGTACV (340)
LYCLDASCL	(341)	ERCPMAKCY (342)
LVCQGSPCL	(343)	QQCQDPYCL* (344)
DXCXDIWCL	(345)	QPCRSMVCA (346)
KTCVGVRV	(347)	WSCHEFMCR (348)
LTCWDWSCR	(350)	SLCRLSTCS (351)
KTCAGSSCI	(352)	VICTGRQCG (353)
NPCFGLLV	(354)	SLCTAFNCH (355)
RTCTPSRCM	(356)	QSCLWRICI (357)
QYCWSKGCR	(358)	LGCFPSWCG (359)

TABLE 9 (cont.)

	VTCSSEWCL	(360)	RLCSWGGCA	(361)
	STCISVHCS	(362)	EVCLVLSCQ	(363)
	IACDGYLCG	(364)	RDCVKNLCR	(365)
5	XGCYQKRCT	(366)	LGCFXSWCG	(367)
	IRCWGGRCS	(368)	IPCSLLGCA	(369)
	AGCVQSQCY	(370)	PRCWERVCS	(371)
	KACFGADCX	(372)	TLCPLVACE	(373)
	SACWLSNCA	(374)	SECYTGSCP	(375)
10	GLCQEHRCW	(376)	VECGFSAVF	(377)
	EDCREWGCR	(378)	HWCRLLACR	(379)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

15 X = Not known.

TABLE 10

PEPTIDES FROM PHAGE RECOVERED FROM ADRENAL GLAND

HKGQVYS	(380)	FSDVHFW*	(381)
RGIFVSS	(382)	PKVKLSE	(383)
LRFWQES	(384)	IWTVVGQ	(385)
DKVGLSV	(386)	SETWRQF	(387)
LDGMIVK	(388)	RYPLAGG	(389)
FTDGEDK	(390)	RSTEHMS	(391)
SGRRHEL	(392)	LMLPRAD*	(27)
SSSRVRS	(393)	YHRSVGR	(394)
PLLRPPH	(395)	SDKLGFV*	(396)
LPRYLLS	(28)	AGSRTNR	(397)
ITQLHKT	(398)	ARCLVYR	(399)
GYVAVMT*	(400)	GLQVKWV	(401)
IFTPGWL	(402)	KQTSRFL	(403)
R(Y/F)LLAGG	(404)		

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

TABLE 11

PEPTIDES FROM PHAGE RECOVERED FROM LIVER

5

ARRGWTL	(405)	SRRFVGG*	(406)
QLTGGCL	(407)	ALERRSL	(408)
10 KAYFRWR	(409)	RWLAWTV	(410)
VGSFIYS*	(411)	LSLLGIA	(412)
LSTVLWF	(413)	SLAMRDS	(414)
GRSSLAC	(415)	SELLGDA	(416)
CGGAGAR	(417)	WRQNMPL*	(418)
15 DFLRCRV	(419)	QAGLRCH	(420)
RALYDAL	(421)	WVSVLGF	(422)
GMAVSSW	(423)	SWFFLVA	(424)
WQSVVRV	(425)	VKSVCRT*	(12)
CGNGHSC	(426)	AEMEGRD	(427)
20 SLRPDNG	(428)	PAMGLIR	(429)

Parentheses contain SEQ ID NO:.

* indicates sequences isolated more than once.

We claim:

       1. A method of identifying a MDP-binding homing molecule that selectively homes to lung endothelium, comprising:

       (a) contacting membrane dipeptidase (MDP) with one or more molecules; and

       (b) determining specific binding of a molecule to said MDP, wherein the presence of specific binding identifies said molecule as a MDP-binding homing molecule that selectively homes to lung endothelium.

       2.   The method of claim 1, wherein said MDP is substantially purified.

       3.   The method of claim 2, wherein said substantially purified MDP is immobilized to a support.

       4.   The method of claim 1, wherein said MDP is human MDP having SEQ ID NO: 448.

       5. A method of selectively directing a moiety to lung endothelium in a subject, comprising administering to said subject a conjugate comprising a moiety linked to a MDP-binding homing molecule identified by the method of claim 1, whereby said moiety is selectively directed to lung endothelium in said subject.

6. The method of claim 5, wherein said MDP-binding homing molecule is a peptide comprising the sequence:

$$X_1-G-F-E-X_2 \text{ (SEQ ID NO: 17)}$$
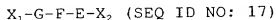
5   wherein X_1 and X_2 each is 1 to 10 independently selected amino acids.

7. The method of claim 6, wherein said MDP-binding homing peptide comprises a sequence selected from the group consisting of CGFECVRQCPERC
10 (SEQ ID NO: 1) and CGFELETC (SEQ ID NO: 2).

8. The method of claim 5, wherein said MDP-binding homing molecule comprises the following Structure 1:
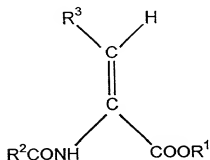
15 wherein R^2 and R^3 are hydrocarbon radicals in the range respectively of 3-10 and 1-15 carbon atoms; in either one of these R^2 or R^3 hydrocarbon chains 1-6 hydrogens may be replaced by halogens or a nonterminal
20 methylene may be replaced by oxygen or sulfur, including oxidized forms of the latter; additionally, a terminal hydrogen in R^3 can also be replaced by hydroxyl or thiol, which

may be acylated or carbamoylated; or the
hydrogen can be replaced by amino, which may
be derivatized as in an acylamino, ureido,
amidino, quanidino, or alkyl or substituted
5 amino group, including quaternary nitrogen
grouping; or, there may be replacement by acid
groups such as carboxylic, phosphonic or
sulfonic acid groups or esters or amides
thereof, or cyano; or combinations thereof,
10 such as a terminal amino acid grouping; and R^1
is hydrogen or lower alkyl (C_{1-6}) or
dialkylaminoalkyl, or a pharmaceutically
acceptable cation.

      9.   The method of claim 8, wherein
15 said MDP-binding homing molecule is 7-(L-2-
amino-2-carboxyethylthio)-2-(2,2-
dimethylcyclopropane carboxamido)-2-heptenoic
acid.

      10.   The method of claim 8, wherein
20 R^2 is branched alkyl or cycloalkyl with a
limitation that the carbon adjacent to the
carbonyl cannot be tertiary.

      11.   The method of claim 10, wherein
R^3 is n-alkyl (1-9 carbons) or n-alkyl (1-9
25 carbons) having a terminal substituent which
is a quaternary nitrogen, amine derivative or
amino acid derived group.

      12.   The method of claim 11, wherein
R^2 is 2,2-dimethylcyclopropyl or
30 2,2-dichlorocyclopropyl and R^3 is a
hydrocarbon chain of 3 to 7 carbon atoms
without a terminal substituent or having a

terminal substituent which is
trimethylammonium, amidino, guanidino or
2-amino-2-carboethylthio.

13. The method of claim 12, wherein
said MDP-binding homing molecule is selected
from the group consisting of:

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-
trimethylammonium hydroxide-2-octenoic acid
inner salt;

Z-2-(2,2-dichlorocyclopropanecarboxamido)-8-
trimethylammonium hydroxide-2-octenoic acid
inner salt;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-
guanidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-
guanidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-
ureido-2-octenoic acid;

Z-8-(1-2-amino-2-carboxyethylthio)-2-(2,2-
dimethylcyclopropanecarboxamido)-2-octenoic
acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-
octenoic acid (racemic and dextrorotatory
forms);

Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-
octenoic acid;

7-(L-2-amino-2-carboxyethylthio)-2-(2,2-
dimethylcyclopropanecarboxamido)-2-heptenoic
acid; and

6-(L-2-amino-2-carboxyethylthio)-2-(2,2-
5   dimethylcyclopropanecarboxamido)-2-hexenoic
acid.

14.   The method of claim 5, wherein said
moiety is a gene therapy vector.

10   15.   The method of claim 5, wherein said
moiety is a drug.

16.   A method of reducing or preventing
lung metastasis in a subject having cancer,
comprising administering to said subject a membrane
15   dipeptidase (MDP)-binding homing molecule.

17.   The method of claim 16, wherein said
MDP-binding homing molecule is a lung homing peptide
comprising the sequence:

X_1-G-F-E-X_2 (SEQ ID NO: 17)

20   wherein X_1 and X_2 each is 1 to 10
independently selected amino acids.

18.   The method of claim 17, wherein said
MDP-binding homing peptide comprises a sequence
selected from the group consisting of CGFECVRQCPERC
25   (SEQ ID NO: 1) and CGFELETC (SEQ ID NO: 2).

19.   The method of claim 18, wherein said
MDP-binding homing peptide is a peptide selected from
the group consisting of CGFECVRQCPERC (SEQ ID NO: 1)

and CGFELETC (SEQ ID NO: 2).

20. The method of claim 16, wherein said MDP-binding homing molecule comprises the following Structure 1:

5

$$R^3 \diagdown \underset{\diagup}{C} \diagdown H$$

$$\underset{R^2CONH \diagup}{\overset{\parallel}{C}} \diagdown COOR^1$$

wherein R^2 and R^3 are hydrocarbon radicals in the range respectively of 3-10 and 1-15 carbon atoms; in either one of these R^2 or R^3 hydrocarbon chains 1-6 hydrogens may be

10 replaced by halogens or a nonterminal methylene may be replaced by oxygen or sulfur, including oxidized forms of the latter; additionally, a terminal hydrogen in R^3 can also be replaced by hydroxyl or thiol, which

15 may be acylated or carbamoylated; or the hydrogen can be replaced by amino, which may be derivatized as in an acylamino, ureido, amidino, quanidino, or alkyl or substituted amino group, including quaternary nitrogen

20 grouping; or, there may be replacement by acid groups such as carboxylic, phosphonic or sulfonic acid groups or esters or amides thereof, or cyano; or combinations thereof, such as a terminal amino acid grouping; and R^1

25 is hydrogen or lower alkyl (C_{1-6}) or dialkylaminoalkyl, or a pharmaceutically

acceptable cation.

21. The method of claim 20, wherein said MDP-binding homing molecule is 7-(L-2-amino-2-carboxyethylthio)-2-(2,2-dimethylcyclopropane carboxamido)-2-heptenoic acid.

22. The method of claim 20, wherein R^2 is branched alkyl or cycloalkyl with a limitation that the carbon adjacent to the carbonyl cannot be tertiary.

23. The method of claim 22, wherein R^3 is n-alkyl (1-9 carbons) or n-alkyl (1-9 carbons) having a terminal substituent which is a quaternary nitrogen, amine derivative or amino acid derived group.

24. The method of claim 23, wherein R^2 is 2,2-dimethylcyclopropyl or 2,2-dichlorocyclopropyl and R^3 is a hydrocarbon chain of 3 to 7 carbon atoms without a terminal substituent or having a terminal substituent which is trimethylammonium, amidino, guanidino or 2-amino-2-carboethylthio.

25. The method of claim 24, wherein said MDP-binding homing molecule is selected from the group consisting of:

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-trimethylammonium hydroxide-2-octenoic acid inner salt;

Z-2-(2,2-dichlorocyclopropanecarboxamido)-8-
trimethylammonium hydroxide-2-octenoic acid
inner salt;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-
5   guanidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-
guanidino-2-octenoic acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-8-
ureido-2-octenoic acid;

10   Z-8-(l-2-amino-2-carboxyethylthio)-2-(2,2-
dimethylcyclopropanecarboxamido)-2-octenoic
acid;

Z-2-(2,2-dimethylcyclopropanecarboxamido)-2-
octenoic acid (racemic and dextrorotatory
15   forms);

Z-2-(2,2-dichlorocyclopropanecarboxamido)-2-
octenoic acid;

7-(L-2-amino-2-carboxyethylthio)-2-(2,2-
dimethylcyclopropanecarboxamido)-2-heptenoic
20   acid; and

6-(L-2-amino-2-carboxyethylthio)-2-(2,2-
dimethylcyclopropanecarboxamido)-2-hexenoic
acid.

        26.   The method of claim 16, wherein
25   said MDP-binding homing molecule is an MDP
inhibitor.

27. The method of claim 26, wherein said MDP inhibitor exhibits a Ki against MDP of at most 1000 nM.

28. The method of claim 27, wherein said MDP inhibitor exhibits a Ki against MDP of at most 100 nM.

29. The method of claim 28, wherein said MDP inhibitor exhibits a Ki against MDP of at most 1 nM.

30. The method of claim 16, wherein said cancer is melanoma.

31. A method of reducing or preventing lung metastasis in a subject having cancer, comprising administering to said subject a membrane dipeptidase (MDP) negative regulatory factor.

32. The method of claim 31, wherein said MDP negative regulatory factor is a soluble MDP polypeptide.

33. The method of claim 31, wherein said MDP negative regulatory factor is an antibody that selectively reacts with MDP.

34. A method of reducing or preventing cell homing to lung endothelium in a subject, comprising administering to said subject a membrane dipeptidase (MDP) negative regulatory factor.

35. The method of claim 34, wherein said MDP negative regulatory factor is a soluble MDP polypeptide.

36. The method of claim 34, wherein said MDP negative regulatory factor is an antibody that selectively reacts with MDP.

37. A method of identifying a molecule that reduces or prevents lung metastasis, comprising the steps of:

(a) contacting membrane dipeptidase (MDP) with one or more molecules; and

(b) determining MDP activity in the presence of said molecule as compared to a control value,

wherein diminished MDP activity in the presence of said molecule identifies said molecule as a molecule that reduces or prevents lung metastasis.

38. The method of claim 37, wherein said MDP is substantially purified.

39. The method of claim 37, wherein MDP activity is determined by release of D-Phe from Gly-D-Phe.

40. The method of claim 37, further comprising the steps of:

(c) administering said molecule to a subject having cancer; and

(d) assaying lung metastasis in said subject as compared to a control level of metastasis.

ABSTRACT OF THE INVENTION

METHODS OF IDENTIFYING LUNG HOMING MOLECULES
USING MEMBRANE DIPEPTIDASE

The present invention provides a method of
5   identifying a membrane dipeptidase (MDP)-binding
homing molecule that selectively homes to lung
endothelium. The method includes the steps of
contacting MDP with one or more molecules; and
determining specific binding of a molecule to the
10 MDP, where the presence of specific binding
identifies the molecule as a MDP-binding homing
molecule that selectively homes to lung endothelium.
Such MDP-binding homing molecules can be linked to a
moiety and, when administered to a subject as a
15 conjugate, can selectively direct the moiety to lung
endothelium in the subject.
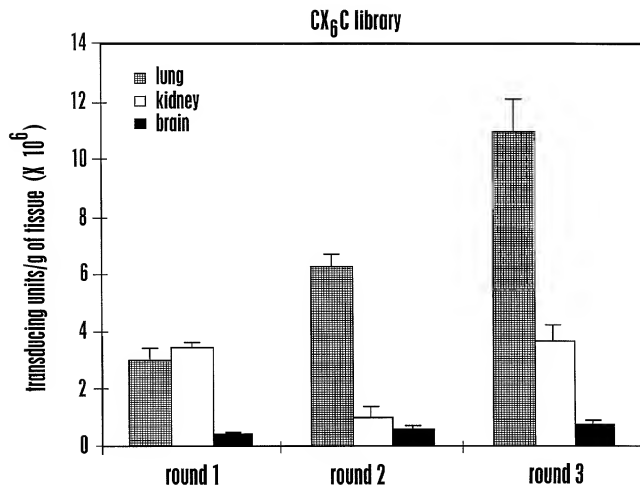
Figure 1
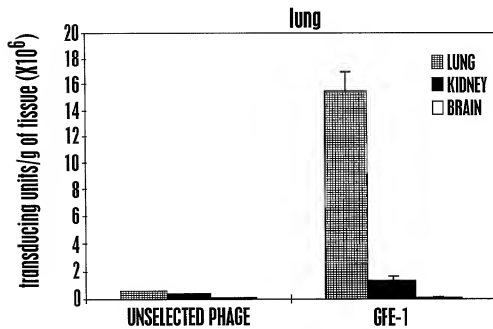
Figure 2A
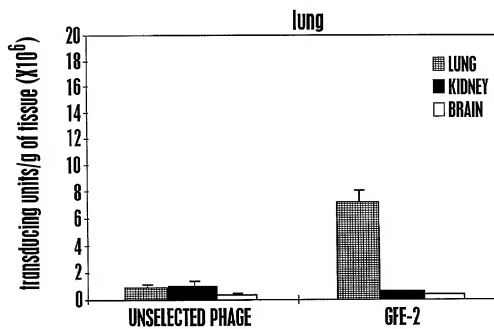
Figure 2B
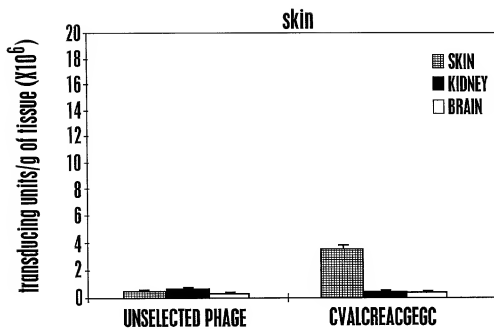
Figure 2C
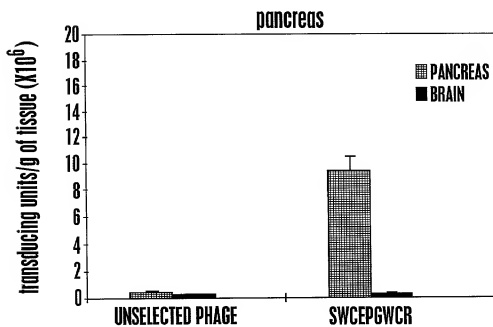
Figure 2D
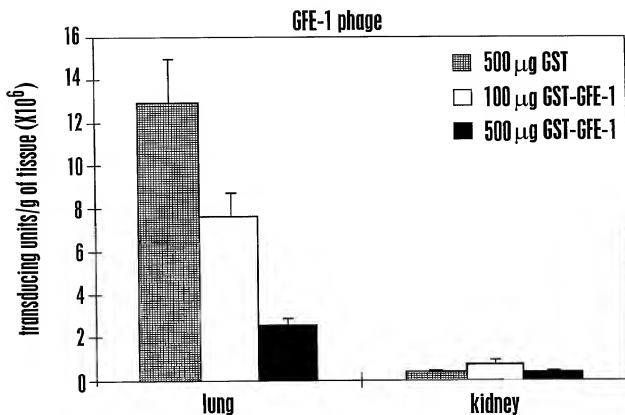
Figure 3A
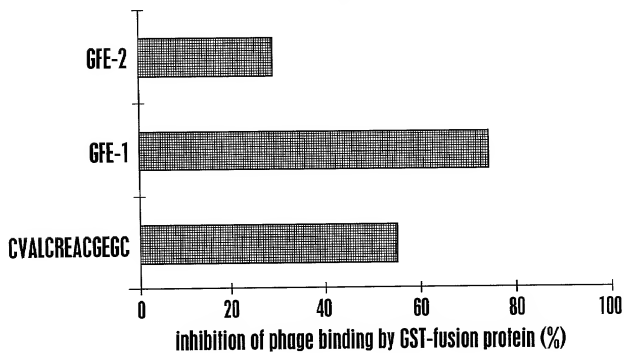
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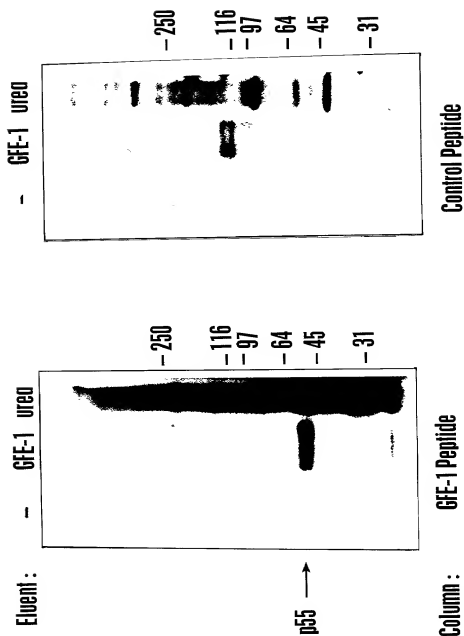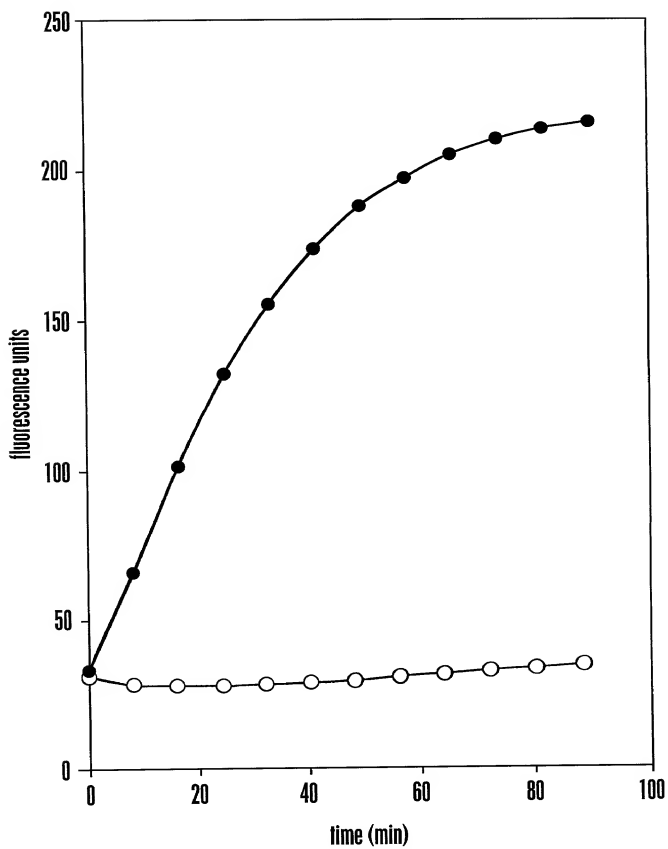
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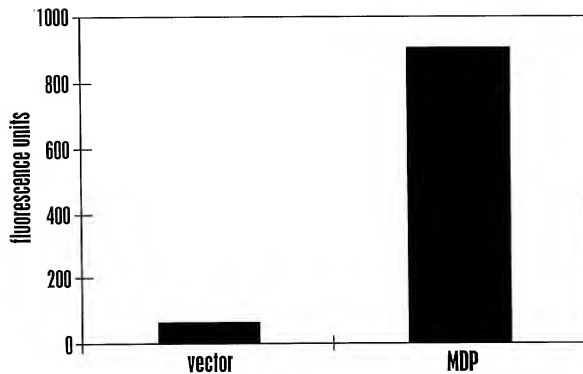
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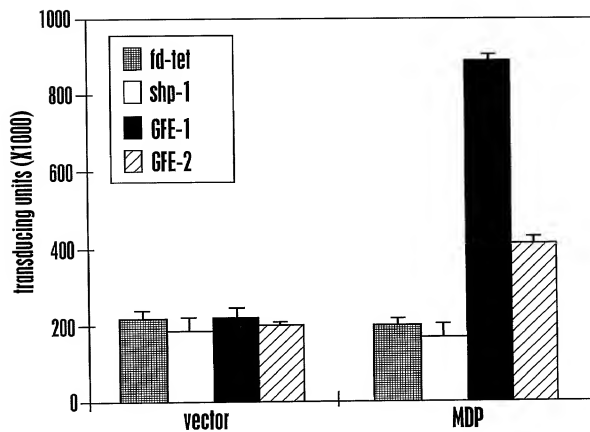
Figure 5

Figure 6A

Figure 6B
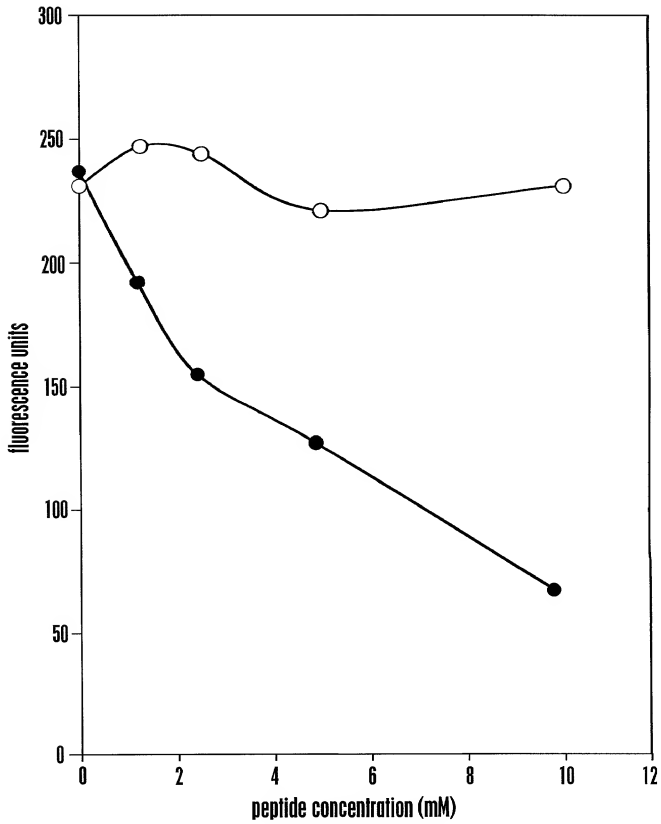
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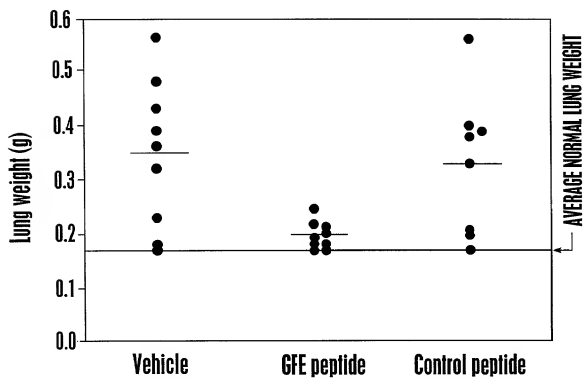
Figure 8

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       -16                                                                                          84
        |                                                                                           |
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PIG  MUTSWWLWPLVAVCAADQFRDLAVRIMQDTPVIDGHNDLPWQLLNLFNNQLQDPGANLSSLAHTHTNIPKLRAGFVGGQFWSAYVPCDTQNRDAVKRTLE
RAT  MLIIWFWSLLAICASDSFRNQAENEMRTTPVIDGHNDLPWQMLTLFENNQLRKSEANLSALAETHTNIPKLRAGFVGGQFWSAYMPCDTQNKDAVKRILE
MOU  MVIIWFWSLLAICASDSFRDQAVAIMRTTPVTDGHNDLPWQLLNLFNNQLLRPDADLNKLAQTHTNIPKLKAGFVGGQFWSAYMPCDTQNKDAVKRILE
RAB  MUTSWWLWPLVAVCTADSFLDQAVQILRVTPVIDGHNDLPWQLLNKFNNRLQDSRANLTVLADTHTNIPKLRAGFVGGQFWSAYTPCDTQNKDTVRRTLE


       1                                        *                                                  184
       |                                        |                                                   |
HUM  QMDVVHRMCRMYPETFLYVTSSAGIRQAFREGKVASLIGVEGGHSIDSSLGVLRALYQLGMRYLTLTHSCNTPWADNULVDTGDSEPQASQGLSPFGQRVV
PIG  QIDVIQRMCQAYPETFACVTSSTGIRQAFREGKVASLVGVEGGHSIDSSLGVLRALYHLGMRYMTLTHSCNTPWADNULVDTGDDKAQSQGLSHFGQSVV
RAT  QIDVIHRMCQLYPETFECVTNSSDILQAFRRGKVASLIGVEGGHLIDSSLGVLRTLYHLGMRYLTLTHNCNTPWADNULVDKGDDEAESQGLSPFGKLVL
MOU  QMDVIHRMCQLYPETFMCVTNSSDILQAFRRGKVASLIGVEGGHLIDSSLGVLRTLYHLGMRYLTLTHNCNTPWADNULVRGDDEAESHGLSPFGKRLL
RAB  QMDVVHRMCQLYPETFLCVTDSAGIQQAFREGKVASLIGVEGGHSIDSSLGVLRALYRLGMRYLTLTHNCNTPWADNULVDRGDDEAQSGGLSVFGQRVV
```

Figure 9A

```
                                                                                            284
                                                                                             |
                                                                      *
HUM  KELNRLGVLIDLAHVSVATMKATLQLSRAPVIFS[G]SAYSVCASRRNVPDDVLRLVKQTDSLVMVNFYNNYISCTNKANLSQVQADHLDHIKEVAGARAVG
PIG  KEMNRLGVMIDLAHVSVATMRAALKLSQAPVIFSHSSAYSLCPHRRNVPDDVLQLVKETGSLVMVNFYNDYVSCSAKANLSQVQADHLDHIKKVAGAAAVG
RAT  NEMNRLGVMIDLSHVSVATMKDALQLVKSTNSLSKAPVIFSHSSAYSVCPHRRNVPDDVLQLVKSTNSLVMVNFYNQFVSCSDSATLSQVQADHLDHIKKVVAGAGAVG
MOU  NEMTRLGVMIDLSHVSVATMKDALQISRAPVIFSHSSAYSLCPHRRNVPDDVLQLVKNTSSLVMVNFFSNFVSCSDSATLPQVQADHLDHIKKVVAGAVG
RAB  REMNRLGVMIDLAHVSVATMKAALQLSTAPVIFSHSSAFTVCAHKRNVPDDVLQLVKETGSLVMVNFYNDYVSCASEATLSQVQADHLDYIKNVAGAAAVR
```

```
                                                          *
                                                                                                    |               411
                                                                                                    ↓                |
HUM  FGGDFGVPRVPEGLEDVSKYPDLIAELLRRNWTEAEVKGALADNLLRVFEAVEQASNLTQAPEEEPIPLDQLGGSCRTHYGYSSGASSLHRHWGLLLASLAPLVLCLSLL
PIG  FGGDYDGVSRVPSGLEDVSKYPDLVAELLRRQWTEAEVRGALADNLLRVFEAVEQASNHAQVPGEEPIPLGQLEASCRTNYGY-SAAPSLHLPPGSLLASLVPLLL-LSLP
RAT  LGGDYDGVTNLPVGLEDVSKYPDLIAELLRRNWTETEVRGLLAENLLRVFSAVELVSNIMQVPEEETIPVEKLDGSCRTFYGH-SRAPSIHLQIGALLASLASLVFSLHPL
MOU  LGGDYDGVTTNLPVGLEDVSKYPDLIAELLRRNWTEVRGLLADNLIRVFSEVELVSSNMAGSPEEVPITLKELDGSCRTYYGY-SQAHSIHLQTGALVASLASLLFRLHLL
RAB  FGGDFGVTRLPVGLEDVSKYPDLVAELLRGWTEAEVRGALADNLIRVFREVEQVSNQAAQVPEEEPISLEQLGGSCRTQYGY-SEAPSLHRRPGCALLASLSLLLLSLGLL
```

Figure 9B

DECLARATION FOR PATENT APPLICATION

As the below-named inventors, we hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled METHODS OF IDENTIFYING LUNG HOMING MOLECULES USING MEMBRANE DIPEPTIDASE, the specification of which

    _____ is attached hereto as Attorney Docket No. _____).

    __XX___ was filed on February 26, 1999, as Application Serial No. 09/258,754 (Attorney Docket No. P-LJ 3443)

and was amended on (or amended through) _____.
                                                              (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to myself to be material to patentability as defined in Title 37, Code of Federal Regulations, Sec. 1.56.

Under Sec. 1.56, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and (1)

It establishes, by itself or in combination with other
information, a prima facie case of unpatentability of a claim;
or (2) It refutes, or is inconsistent with, a position the
applicant takes in: (I) Opposing an argument of
unpatentability relied on by the U.S. Patent and Trademark
Office, or (ii) Asserting an argument of patentability.

I hereby claim the benefit under Title 35,
United States Code, §120 of any United States application(s)
listed below and, insofar as the subject matter of each of the
claims of this application is not disclosed in the prior
United States application in the manner provided by the first
paragraph of Title 35, United States Code §112, I acknowledge
the duty to disclose material information as defined in Title
37, Code of Federal Regulations, §1.56(a) which occurred
between the filing date of the prior application(s) and the
national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status
09/042,107	March 13, 1998	Pending

I hereby declare that all statements made
herein of my own knowledge are true and that all statements
made on information and belief are believed to be true; and
further that these statements were made with the knowledge
that willful false statements and the like so made are
punishable by fine or imprisonment, or both, under Section
1001 of Title 18 of the United States Code and that such
willful false statements may jeopardize the validity of the
application or any patent issued thereon.

Full name of first inventor: Daniel Rajotte

Inventor's signature: _____

Date: _____March 26, 1999_____

Residence: Del Mar, California, USA

Citizenship: Canada

Post Office Address: 517½ Stratford Court
                        Del Mar, CA, 92014 USA

Full name of second inventor: Renata Pasqualini

Inventor's signature: _____

Date: _____4/14/99_____

Residence: Solana Beach, California, USA

Citizenship: Brazil

Post Office Address: 707 S. Sierra Ave., #29
                        Solana Beach, CA, 92075 USA

Full name of third inventor: Erkki Ruoslahti

Inventor's signature: _____

Date: _____4-15-99_____

Residence: Rancho Santa Fe, California, USA

Citizenship: USA

Post Office Address: Post Office Box 1054
                        Rancho Santa Fe, CA, 92067 USA

<110> Ruoslahti, Erkki
     Pasqualini, Renata
     Rajotte, Daniel

<120> Methods of Identifying Lung Homing Molecules Using
     Membrane Dipeptidase

<130> P-LJ 4377

<140>
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<150> 09/042,107
<151> 1998-03-13

<160> 452

<170> PatentIn Ver. 2.0

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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 1   5

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<210> 68
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<220>
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Cys Thr Gly His Leu Ser Thr Asp Cys
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<400> 72
Cys Gln Asp Leu Asn Ile Met Gln Cys
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<210> 73
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<400> 73
Thr Arg Arg Thr Asn Asn Pro Leu Thr
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<220>
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<400> 74
Cys Gly Tyr Ile Asp Pro Asn Arg Ile Ser Gln Cys
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<400> 75
Cys Thr Val Asn Glu Ala Tyr Lys Thr Arg Met Cys
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<220>
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<400> 76
Cys Arg Leu Arg Ser Tyr Gly Thr Leu Ser Leu Cys
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<210> 77
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<220>
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<400> 77
Cys Ala Gly Thr Cys Ala Thr Gly Cys Asn Gly Val Cys
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<210> 78
<211> 12
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<223> Description of Artificial Sequence: Synthetic

<400> 78
Cys Ala Asp Tyr Asp Leu Ala Leu Gly Leu Met Cys
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<210> 79
<211> 12
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 79
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<210> 80
<211> 12
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<220>
<223> Description of Artificial Sequence: Synthetic

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<220>
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<400> 81
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<220>
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<400> 82
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<220>
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<400> 83
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<400> 84
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<400> 85
Cys Trp Glu Glu His Pro Ser Ile Lys Trp Trp Cys
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<210> 86
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<400> 86
Cys Trp Asp Ala Asp Gln Ile Phe Gly Ile Lys Cys
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<210> 87
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<400> 87
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  1   5   10

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<400> 88
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  1   5   10

<210> 89
<211> 12
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<400> 89
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  1   5   10

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  1   5   10

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<211> 12
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<400> 91
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  1   5   10

<210> 92
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<220>
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<400> 92
Cys Asn Ser Lys Ser Ser Ala Glu Leu Glu Lys Cys
  1   5   10

<210> 93
<211> 12
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<220>
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<400> 93
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  1   5   10

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<220>
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<400> 94
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<210> 95
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<220>
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<400> 95
Cys Glu Gly His Ser Met Arg Gly Tyr Gly Leu Cys
  1               5                   10


<210> 96
<211> 12
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<220>
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<400> 96
Cys Arg Asp Arg Gly Asp Arg Met Lys Ser Leu Cys
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<210> 97
<211> 12
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<220>
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<400> 97
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Cys Asp Asn Thr Cys Thr Tyr Gly Val Asp Asp Cys
  1   5   10

<210> 98
<211> 12
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 98
Cys Ser Ala His Ser Gln Glu Met Asn Val Asn Cys
  1   5   10

<210> 99
<211> 13
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<220>
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<400> 99
Cys Gly Ala Ala Cys Gly Val Gly Cys Arg Gly Arg Cys
  1   5   10

<210> 100
<211> 13
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<220>
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<400> 100
Cys Leu Val Gly Cys Arg Leu Ser Cys Gly Gly Glu Cys
  1   5   10

<210> 101
<211> 13
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<400> 101
Cys Arg Ser Gly Cys Val Glu Gly Cys Gly Gly Arg Cys
 1   5   10

<210> 102
<211> 13
<212> PRT
<213> Artificial Sequence

<400> 102
Cys Ile Ala Arg Cys Gly Gly Ala Cys Gly Arg His Cys
 1   5   10

<210> 103
<211> 13
<212> PRT
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<400> 103
Cys Gly Gly Glu Cys Gly Trp Glu Cys Glu Val Ser Cys
 1   5   10

<210> 104
<211> 13
<212> PRT
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<400> 104
Cys Gly Val Gly Cys Pro Gly Leu Cys Gly Gly Ala Cys
 1   5   10

<210> 105

<211> 13
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 105
Cys Lys Trp Leu Cys Leu Leu Leu Cys Ala Val Ala Cys
 1   5   10

<210> 106
<211> 13
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 106
Cys Ser Glu Gly Cys Gly Pro Val Cys Trp Pro Glu Cys
 1   5   10

<210> 107
<211> 13
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 107
Cys Gly Ala Ala Cys Gly Val Gly Cys Gly Gly Arg Cys
 1   5   10

<210> 108
<211> 13
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<220>
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<400> 108
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  1                5                    10
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<210> 109
<211> 13
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 109

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<210> 110
<211> 13
<212> PRT
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<220>
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<400> 110

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  1                5                    10
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<210> 111
<211> 13
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<220>
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<400> 111

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  1                5                    10
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<210> 112
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<220>

<400> 112
Cys Tyr Trp Trp Cys Asp Gly Val Cys Ala Leu Gln Cys
  1   5   10

<210> 113
<211> 13
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<220>

<400> 113
Cys Ala Gly Gly Cys Ala Val Arg Cys Gly Gly Thr Cys
  1   5   10

<210> 114
<211> 13
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<220>

<400> 114
Cys Gly Gly Ala Cys Gly Gly Val Cys Thr Gly Gly Cys
  1   5   10

<210> 115
<211> 13
<212> PRT
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<220>

<400> 115
Cys Gly Arg Pro Cys Val Gly Glu Cys Arg Met Gly Cys
  1   5   10

<210> 116
<211> 13

33

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<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 116
Cys Leu Val Gly Cys Glu Val Gly Cys Ser Pro Ala Cys
  1               5                   10


<210> 117
<211> 13
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 117
Cys Pro Arg Thr Cys Gly Ala Ala Cys Ala Ser Pro Cys
  1               5                   10


<210> 118
<211> 13
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 118
Cys Arg Gly Asp Cys Gly Ile Gly Cys Arg Arg Leu Cys
  1               5                   10


<210> 119
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 119
Glu Ile Cys Gln Leu Gly Ser Cys Thr
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<210> 120
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 120
Trp Arg Cys Glu Gly Phe Asn Cys Gln
  1               5


<210> 121
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 121
Arg Lys Cys Leu Arg Pro Asp Cys Gly
  1               5


<210> 122
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 122
Leu Ala Cys Phe Val Thr Gly Cys Leu
  1               5


<210> 123
<211> 9
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<220>
<223> Description of Artificial Sequence: Synthetic
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<400> 123
Gly Leu Cys Asn Gly Ala Thr Cys Met
  1   5

<210> 124
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 124
Asp Met Cys Trp Leu Ile Gly Cys Gly
  1   5

<210> 125
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 125
Ser Gly Cys Arg Thr Met Val Cys Val
  1   5

<210> 126
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 126
Gln Arg Cys Pro Arg Ser Phe Cys Leu
  1   5

<210> 127
<211> 9
<212> PRT

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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 127
Leu Ser Cys Ala Pro Val Ile Cys Gly
 1   5

<210> 128
<211> 9
<212> PRT
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<220>
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<400> 128
Arg Glu Cys Thr Asn Glu Ile Cys Tyr
 1   5

<210> 129
<211> 9
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<213> Artificial Sequence

<220>
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<400> 129
Asn Glu Cys Leu Met Ile Ser Cys Arg
 1   5

<210> 130
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
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<400> 130
Ser Cys Val Phe Cys Asp Trp Leu Ser
 1   5

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<210> 131
<211> 9
<212> PRT
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<220>
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<400> 131
Trp Ala Cys Glu Glu Leu Ser Cys Phe
  1               5


<210> 132
<211> 9
<212> PRT
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<400> 132
Gln Asn Cys Pro Val Thr Arg Cys Val
  1               5


<210> 133
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 133
Cys Ala Thr Leu Thr Asn Asp Glu Cys
  1               5


<210> 134
<211> 8
<212> PRT
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<220>
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<400> 134
Cys Asp Asn Arg Glu Met Ser Cys
  1   5

<210> 135
<211> 8
<212> PRT
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<220>
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<400> 135
Cys Phe Met Asp His Ser Asn Cys
  1   5

<210> 136
<211> 8
<212> PRT
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<220>
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<400> 136
Cys Gly Glu Tyr Gly Arg Glu Cys
  1   5

<210> 137
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 137
Cys His Met Lys Arg Asp Arg Thr Cys
  1   5

<210> 138
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 138
Cys Lys Lys Arg Leu Leu Asn Val Cys
  1   5

<210> 139
<211> 8
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 139
Cys Leu Asp Tyr His Pro Lys Cys
  1   5

<210> 140
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 140
Cys Met Thr Gly Arg Val Thr Cys
  1   5

<210> 141
<211> 8
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 141
Cys Asn Lys Ile Val Arg Arg Cys
  1   5

<210> 142
<211> 8
<212> PRT
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<220>
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<400> 142
Cys Pro Asp Leu Leu Val Ala Cys
  1   5

<210> 143
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 143
Cys Ser Asp Thr Gln Ser Ile Gly Cys
  1   5

<210> 144
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 144
Cys Ser Lys Ala Tyr Asp Leu Ala Cys
  1   5

<210> 145
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 145

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Cys Ser Lys Lys Gly Pro Ser Tyr Cys
  1                   5


<210> 146
<211> 9
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 146
Cys Thr Leu Lys His Thr Ala Met Cys
  1                   5


<210> 147
<211> 8
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 147
Cys Thr Gln His Ile Ala Asn Cys
  1                   5


<210> 148
<211> 8
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 148
Cys Thr Thr Glu Ile Asp Tyr Cys
  1                   5


<210> 149
<211> 9
<212> PRT
<213> Artificial Sequence
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42

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 149
Cys Val Gly Arg Ser Gly Glu Leu Cys
  1   5

<210> 150
<211> 7
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 150
Tyr Ala Gly Phe Phe Leu Val
  1   5

<210> 151
<211> 7
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 151
Arg Ser Gly Ala Arg Ser Ser
  1   5

<210> 152
<211> 7
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 152
Cys Val Glu Ser Thr Val Ala
  1   5

<210> 153

<211> 7
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 153
Ser Arg Arg Gln Pro Leu Ser
  1   5

<210> 154
<211> 7
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 154
Ser Lys Val Trp Leu Leu Leu
  1   5

<210> 155
<211> 7
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 155
Gln Val Arg Arg Val Pro Glu
  1   5

<210> 156
<211> 7
<212> PRT
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<220>
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<400> 156
Tyr Ser Gly Lys Trp Gly Trp

<210> 157
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 157
Met Val Gln Ser Val Gly
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<210> 158
<211> 7
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 158
Leu Arg Ala Val Gly Arg Ala
  1   5

<210> 159
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
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<400> 159
Met Ser Pro Gln Leu Ala Thr
  1   5

<210> 160
<211> 7
<212> PRT
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<220>

<400> 160
Gly Ala Val Leu Pro Gly Glu
  1   5

<210> 161
<211> 7
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<220>

<400> 161
Trp Ile Glu Glu Ala Glu Arg
  1   5

<210> 162
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<220>

<400> 162
Leu Val Ser Glu Gln Leu Arg
  1   5

<210> 163
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<220>

<400> 163
Arg Gly Asp Arg Pro Pro Tyr
  1   5

<210> 164
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<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 164
Val Arg Arg Gly Ser Pro Gln
  1                 5


<210> 165
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 165
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  1                 5


<210> 166
<211> 7
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 166
Gly Ile Ser Ala Val Leu Ser
  1                 5


<210> 167
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 167
Gly Gly Arg Gly Ser Trp Glu
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<210> 168
<211> 7
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 168
Gly Val Ser Ala Ser Asp Trp
  1               5


<210> 169
<211> 7
<212> PRT
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 169
Phe Arg Val Arg Gly Ser Pro
  1               5


<210> 170
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 170
Ser Arg Leu Ser Gly Gly Thr
  1               5


<210> 171
<211> 7
<212> PRT
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<220>
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<400> 171
Trp Glu Leu Val Ala Arg Ser
 1   5

<210> 172
<211> 7
<212> PRT
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<220>
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<400> 172
Met Arg Arg Asp Glu Gln Arg
 1   5

<210> 173
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<220>
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<400> 173
Gly Cys Arg Cys Trp Ala
 1   5

<210> 174
<211> 13
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<213> Artificial Sequence

<220>
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<400> 174
Cys Tyr Ala Asp Cys Glu Gly Thr Cys Gly Met Val Cys
 1   5   10

<210> 175
<211> 13
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 175
Cys Trp Asn Ile Cys Pro Gly Gly Cys Arg Ala Leu Cys
 1   5   10

<210> 176
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 176
Gly Pro Gly Cys Glu Glu Glu Cys Gln Pro Ala Cys
 1   5   10

<210> 177
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 177
Cys Lys Gly Thr Cys Val Leu Gly Cys Ser Glu Glu Cys
 1   5   10

<210> 178
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 178
Cys Ser Thr Leu Cys Gly Leu Arg Cys Met Gly Thr Cys
 1   5   10

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<210> 179
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 179
Cys Met Pro Arg Cys Gly Val Asn Cys Lys Trp Ala Cys
 1               5                   10


<210> 180
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 180
Cys Val Gly Ala Cys Asp Leu Lys Cys Thr Gly Gly Cys
 1               5                   10


<210> 181
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 181
Cys Ser Ser Gly Cys Ser Lys Asn Cys Leu Glu Met Cys
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<210> 182
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<210> 183
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<220>
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<400> 183
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<210> 184
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<220>
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<400> 184
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<220>
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<210> 186
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<220>
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<400> 186
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<210> 187
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<400> 187
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<220>
<223> Description of Artificial Sequence: Synthetic

<400> 188
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<220>
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<400> 189
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<210> 190
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<220>
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<400> 190
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<220>
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<210> 192
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<400> 192
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<210> 193
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<220>
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<400> 193

Cys Pro Ser Asn Cys Val Ala Leu Cys Thr Ser Gly Cys
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<210> 194
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<400> 194
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<210> 195
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<210> 196
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<210> 197
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<220>
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<210> 198
<211> 9
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<220>
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<400> 198
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<220>
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<400> 203
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<210> 211
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<220>
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<210> 212
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<210> 219
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<210> 220
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<400> 225
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<210> 226
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<220>
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<400> 240
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<210> 241
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<400> 241
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<400> 242
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<400> 252
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<210> 261
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<400> 262
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  1   5

<210> 263
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<210> 265
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<220>
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Glu Val Gln Ser Ala Lys Trp
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<210> 266
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<220>
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<400> 266
Lys Arg Val Tyr Val Leu Gly
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<210> 267
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<210> 269
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<210> 270
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<212> PRT
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<400> 271
Gly Phe Tyr Arg Met Leu Gly
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<210> 272
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<220>
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<210> 273
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<220>
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<400> 273
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<210> 274
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<210> 275
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<220>
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<210> 276
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<213> Artificial Sequence

<220>
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<400> 276
Phe Val Trp Leu Val Gly Ser
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<210> 277
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<220>
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<400> 277
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<210> 278
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<212> PRT
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<220>
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Trp His Gln Pro Leu
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<210> 279
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<220>
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<400> 279
Arg Gly Arg Trp Leu Ala Leu
  1               5


<210> 280
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<400> 280
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<210> 281
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Leu Trp Leu Ser Gly Asn Trp
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<210> 282
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<220>
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<210> 283
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<400> 283
Trp Thr Phe Leu Glu Arg Leu
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<210> 284
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<210> 285
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<400> 285
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<210> 286
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<400> 286
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<210> 287
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<400> 287
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<210> 288
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<400> 289

Ser Leu Trp Tyr Leu Gly Ala
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<210> 290
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<210> 291
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<400> 291
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<210> 292
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<220>
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<400> 292
Gly Val Arg Thr Ser Ile Trp
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<210> 293
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<220>
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<400> 293
Arg Pro Val Gly Met Arg Lys
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<210> 294
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<213> Artificial Sequence

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<400> 294
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<210> 295
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<400> 295
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  1   5

<210> 296
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<210> 297

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<400> 297
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<210> 299
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<400> 310
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<400> 313
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<400> 317
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<220>
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<400> 333
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<400> 346
Gln Pro Cys Arg Ser Met Val Cys Ala
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<400> 347
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<210> 348
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Trp Ser Cys His Glu Phe Met Cys Arg
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<400> 356
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<400> 373
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<210> 376
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<210> 378
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<210> 380
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<400> 382
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<210> 389
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<210> 390
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<220>
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<220>
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<210> 392
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<220>
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<210> 393
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<220>
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<400> 393
Ser Ser Ser Arg Val Arg Ser
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<210> 394
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<220>
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<210> 396
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<210> 397
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<400> 402
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 417
Cys Gly Gly Ala Gly Ala Arg
  1               5


<210> 418
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 418
Trp Arg Gln Asn Met Pro Leu
  1               5


<210> 419
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 419
Asp Phe Leu Arg Cys Arg Val
  1               5
```

```
<210> 420
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 420
Gln Ala Gly Leu Arg Cys His
  1               5


<210> 421
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 421
Arg Ala Leu Tyr Asp Ala Leu
  1               5


<210> 422
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 422
Trp Val Ser Val Leu Gly Phe
  1               5


<210> 423
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 423
```

```
Gly Met Ala Val Ser Ser Trp
  1                 5


<210> 424
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 424
Ser Trp Phe Phe Leu Val Ala
  1                 5


<210> 425
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 425
Trp Gln Ser Val Val Arg Val
  1                 5


<210> 426
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 426
Cys Gly Asn Gly His Ser Cys
  1                 5


<210> 427
<211> 7
<212> PRT
<213> Artificial Sequence
```

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 427
Ala Glu Met Glu Gly Arg Asp
  1   5

<210> 428
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 428
Ser Leu Arg Pro Asp Asn Gly
  1   5

<210> 429
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 429
Pro Ala Met Gly Leu Ile Arg
  1   5

<210> 430
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 430
Leu Ala Gly Gly
  1

<210> 431

<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)
<223> 1 to 10 independently selected amino acids

<220>
<221> SITE
<222> (14)..(23)
<223> 1 to 10 independently selected amino acids

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 431
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Pro Arg Xaa Xaa Xaa
 1   5   10   15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            20

<210> 432
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)..(10)
<223> 1 to 10 independently selected amino acids

<220>
<221> SITE
<222> (15)..(25)
<223> 0 to 10 independently selected amino acids

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 432
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Ala Gly Gly Xaa Xaa
 1   5   10   15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

```
<210> 433
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (6)..(15)
<223> 1 to 10 independently selected amino acids

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 433
Tyr Ser Gly Lys Trp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
  1               5                   10                  15


<210> 434
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<221> SITE
<222> (1)..(10)
<223> 1 to 10 independently selected amino acids

<220>
<221> SITE
<222> (14)..(23)
<223> 1 to 10 independently selected amino acids

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 434
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Arg Gly Ser Xaa Xaa Xaa
  1               5                   10                  15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa
              20


<210> 435
```

<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 435
Cys Cys Phe Thr Asn Phe Asp Cys Tyr Leu Gly Cys
  1   5   10

<210> 436
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<221> UNSURE
<222> (1)..(10)
<223> 1 to 10 independently selected amino acids

<220>
<221> UNSURE
<222> (14)..(23)
<223> 1 to 10 independently selected amino acids

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 436
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Arg Asp Val Xaa Xaa Xaa
  1   5   10   15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            20

<210> 437
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      Peptide

<400> 437

122

```
Cys Val Asp Val Cys Cys Asp Gly Cys Pro Val Cys Cys
  1               5                   10
```

<210> 438
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      Peptide

<400> 438
```
Arg Val Pro Leu Ser Gly Asp Val Glu His
  1               5                   10
```

<210> 439
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      Peptide

<400> 439
```
Leu Arg Val Met Ser Phe Thr Ser Gly Gln
  1               5                   10
```

<210> 440
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      Peptide

<400> 440
```
Arg Phe Ser Val Gly Ser Leu Phe Gly Ser
  1               5                   10
```

<210> 441
<211> 13

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      Peptide

<400> 441
Cys Gly Ala Thr Cys Glu Met Gln Cys Pro Ser Gly Cys
  1   5   10

<210> 442
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      Peptide

<400> 442
Gly Arg Gly Glu Ser Pro
  1   5

<210> 443
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      Peptide

<400> 443
Cys Ala Arg Ala Cys
  1   5

<210> 444
<211> 10
<212> PRT
<213> Rattus sp.

<400> 444
Tyr Pro Asp Leu Ile Ala Glu Leu Leu Arg
  1   5   10

<210> 445
<211> 24
<212> PRT
<213> Rattus sp.

<400> 445
Thr Thr Pro Val Ile Asp Gly His Asn Asp Leu Pro Trp Gln Met Leu
1   5   10   15

Thr Leu Phe Asn Asn Gln Leu Arg
            20

<210> 446
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      Nucleotide

<400> 446
ccgctggtac cgcagatccc tggggacctt g   31

<210> 447
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      Nucleotide

<400> 447
tctttctaga gctcagagag cactggagga g   31

<210> 448
<211> 411
<212> PRT
<213> Homo sapiens

<400> 448
Met Trp Ser Gly Trp Trp Leu Trp Ser Leu Val Ala Val Cys Thr Ala
1   5   10   15

```
Asp Phe Phe Arg Asp Glu Ala Glu Arg Ile Met Arg Asp Ser Pro Val
            20                  25                      30

Ile Asp Gly His Asn Asp Leu Pro Trp Gln Leu Leu Asp Met Phe Asn
        35                  40                  45

Asn Arg Leu Gln Asp Glu Arg Ala Asn Leu Thr Thr Leu Ala Gly Thr
    50                  55                  60

His Thr Asn Ile Pro Lys Leu Arg Ala Gly Phe Val Gly Gly Gln Phe
65                  70                  75                      80

Trp Ser Val Tyr Thr Pro Cys Asp Thr Gln Asn Lys Asp Ala Val Arg
                85                  90                      95

Arg Thr Leu Glu Gln Met Asp Val Val His Arg Met Cys Arg Met Tyr
            100                 105                 110

Pro Glu Thr Phe Leu Tyr Val Thr Ser Ser Ala Gly Ile Arg Gln Ala
        115                 120                 125

Phe Arg Glu Gly Lys Val Ala Ser Leu Ile Gly Val Glu Gly Gly His
    130                 135                 140

Ser Ile Asp Ser Ser Leu Gly Val Leu Arg Ala Leu Tyr Gln Leu Gly
145                 150                 155                 160

Met Arg Tyr Leu Thr Leu Thr His Ser Cys Asn Thr Pro Trp Ala Asp
            165                 170                 175

Asn Trp Leu Val Asp Thr Gly Asp Ser Glu Pro Gln Ser Gln Gly Leu
            180                 185                 190

Ser Pro Phe Gly Gln Arg Val Val Lys Glu Leu Asn Arg Leu Gly Val
        195                 200                 205

Leu Ile Asp Leu Ala His Val Ser Val Ala Thr Met Lys Ala Thr Leu
    210                 215                 220

Gln Leu Ser Arg Ala Pro Val Ile Phe Ser His Ser Ser Ala Tyr Ser
225                 230                 235                 240

Val Cys Ala Ser Arg Arg Asn Val Pro Asp Asp Val Leu Arg Leu Val
                245                 250                 255

Lys Gln Thr Asp Ser Leu Val Met Val Asn Phe Tyr Asn Asn Tyr Ile
            260                 265                 270
```

126

```
Ser Cys Thr Asn Lys Ala Asn Leu Ser Gln Val Ala Asp His Leu Asp
         275                 280                 285

His Ile Lys Glu Val Ala Gly Ala Arg Ala Val Gly Phe Gly Gly Asp
     290                 295                 300

Phe Asp Gly Val Pro Arg Val Pro Glu Gly Leu Glu Asp Val Ser Lys
305                 310                 315                 320

Tyr Pro Asp Leu Ile Ala Glu Leu Leu Arg Arg Asn Trp Thr Glu Ala
             325                 330                 335

Glu Val Lys Gly Ala Leu Ala Asp Asn Leu Leu Arg Val Phe Glu Ala
             340                 345                 350

Val Glu Gln Ala Ser Asn Leu Thr Gln Ala Pro Glu Glu Glu Pro Ile
         355                 360                 365

Pro Leu Asp Gln Leu Gly Gly Ser Cys Arg Thr His Tyr Gly Tyr Ser
     370                 375                 380

Ser Gly Ala Ser Ser Leu His Arg His Trp Gly Leu Leu Leu Ala Ser
385                 390                 395                 400

Leu Ala Pro Leu Val Leu Cys Leu Ser Leu Leu
             405                 410
```

<210> 449
<211> 409
<212> PRT
<213> Sus scrofa

<400> 449
```
Met Trp Thr Ser Trp Trp Leu Trp Pro Leu Val Ala Val Cys Ala Ala
  1                 5                 10                 15

Asp Gln Phe Arg Asp Leu Ala Val Arg Ile Met Gln Asp Thr Pro Val
             20                 25                 30

Ile Asp Gly His Asn Asp Leu Pro Trp Gln Leu Leu Asn Leu Phe Asn
         35                 40                 45

Asn Gln Leu Gln Asp Pro Gly Ala Asn Leu Ser Ser Leu Ala His Thr
     50                 55                 60

His Thr Asn Ile Pro Lys Leu Lys Ala Gly Phe Val Gly Gly Gln Phe
65                 70                 75                 80
```

```
Trp Ser Ala Tyr Val Pro Cys Asp Thr Gln Asn Arg Asp Ala Val Lys
                    85                  90                  95

Arg Thr Leu Glu Gln Ile Asp Val Ile Gln Arg Met Cys Gln Ala Tyr
            100             105             110

Pro Glu Thr Phe Ala Cys Val Thr Ser Ser Thr Gly Ile Arg Gln Ala
        115             120             125

Phe Arg Glu Gly Lys Val Ala Ser Leu Val Gly Val Glu Gly Gly His
    130             135             140

Ser Ile Asp Ser Ser Leu Gly Val Leu Arg Ala Leu Tyr His Leu Gly
145             150             155             160

Met Arg Tyr Met Thr Leu Thr His Ser Cys Asn Thr Pro Trp Ala Asp
                165             170             175

Asn Trp Leu Val Asp Thr Gly Asp Asp Lys Ala Gln Ser Gln Gly Leu
            180             185             190

Ser His Phe Gly Gln Ser Val Val Lys Glu Met Asn Arg Leu Gly Val
        195             200             205

Met Ile Asp Leu Ala His Val Ser Val Ala Thr Met Arg Ala Ala Leu
        210             215             220

Lys Leu Ser Gln Ala Pro Val Ile Phe Ser His Ser Ser Ala Tyr Ser
225             230             235             240

Leu Cys Pro His Arg Arg Asn Val Pro Asp Asp Val Leu Gln Leu Val
            245             250             255

Lys Glu Thr Gly Ser Leu Val Met Val Asn Phe Tyr Asn Asp Tyr Val
            260             265             270

Ser Cys Ser Ala Lys Ala Asn Leu Ser Gln Val Ala Asp His Leu Asp
        275             280             285

His Ile Lys Lys Val Ala Gly Ala Ala Ala Val Gly Phe Gly Gly Asp
    290             295             300

Tyr Asp Gly Val Ser Arg Val Pro Ser Gly Leu Glu Asp Val Ser Lys
305             310             315             320

Tyr Pro Asp Leu Val Ala Glu Leu Leu Arg Arg Gln Trp Thr Glu Ala
                325             330             335
```

128

```
Glu Val Arg Gly Ala Leu Ala Asp Asn Leu Leu Arg Val Phe Glu Ala
            340                 345                 350

Val Glu Gln Ala Ser Asn His Ala Gln Val Pro Gly Glu Glu Pro Ile
        355                 360                 365

Pro Leu Gly Gln Leu Glu Ala Ser Cys Arg Thr Asn Tyr Gly Tyr Ser
    370                 375                 380

Ala Ala Pro Ser Leu His Leu Pro Pro Gly Ser Leu Leu Ala Ser Leu
385                 390                 395                 400

Val Pro Leu Leu Leu Leu Ser Leu Pro
                405


<210> 450
<211> 410
<212> PRT
<213> Rattus sp.

<400> 450
Met Leu Ile Ile Trp Trp Phe Trp Ser Leu Leu Ala Ile Cys Ala Ser
  1               5                 10                  15

Asp Ser Phe Arg Asn Gln Ala Glu Asn Ile Met Arg Thr Thr Pro Val
            20                  25                  30

Ile Asp Gly His Asn Asp Leu Pro Trp Gln Met Leu Thr Leu Phe Asn
        35                  40                  45

Asn Gln Leu Arg Lys Ser Glu Ala Asn Leu Ser Ala Leu Ala Glu Thr
    50                  55                  60

His Thr Asn Ile Pro Lys Leu Arg Ala Gly Phe Val Gly Gly Gln Phe
65                  70                  75                  80

Trp Ser Ala Tyr Met Pro Cys Asp Thr Gln Asn Lys Asp Ala Val Lys
                85                  90                  95

Arg Ile Leu Glu Gln Ile Asp Val Ile His Arg Met Cys Gln Leu Tyr
            100                 105                 110

Pro Glu Thr Phe Glu Cys Val Thr Asn Ser Ser Asp Ile Leu Gln Ala
            115                 120                 125

Phe Arg Arg Gly Lys Val Ala Ser Leu Ile Gly Val Glu Gly Gly His
                            129
```

Leu Ile Asp Ser Ser Leu Gly Val Leu Arg Thr Leu Tyr His Leu Gly
145   150   155   160

Met Arg Tyr Leu Thr Leu Thr His Asn Cys Asn Thr Pro Trp Ala Asp
                      165   170   175

Asn Trp Leu Val Asp Lys Gly Asp Asp Glu Ala Glu Ser Gln Gly Leu
                  180   185   190

Ser Pro Phe Gly Lys Leu Val Leu Asn Glu Met Asn Arg Leu Gly Val
              195   200   205

Met Ile Asp Leu Ser His Val Ser Val Ala Thr Met Lys Asp Ala Leu
              210   215   220

Gln Leu Ser Lys Ala Pro Val Ile Phe Ser His Ser Ser Ala Tyr Ser
225   230   235   240

Val Cys Pro His Arg Arg Asn Val Pro Asp Asp Val Leu Gln Leu Val
                  245   250   255

Lys Ser Thr Asn Ser Leu Val Met Val Asn Phe Tyr Asn Gln Phe Val
              260   265   270

Ser Cys Ser Asp Ser Ala Thr Leu Ser Gln Val Ala Asp His Leu Asp
          275   280   285

His Ile Lys Lys Val Ala Gly Ala Gly Ala Val Gly Leu Gly Gly Asp
      290   295   300

Tyr Asp Gly Val Thr Asn Leu Pro Val Gly Leu Glu Asp Val Ser Lys
305   310   315   320

Tyr Pro Asp Leu Ile Ala Glu Leu Leu Arg Arg Asn Trp Thr Glu Thr
                  325   330   335

Glu Val Arg Gly Leu Leu Ala Glu Asn Leu Leu Arg Val Phe Ser Ala
              340   345   350

Val Glu Leu Val Ser Asn Ile Met Gln Val Pro Glu Glu Thr Ile
          355   360   365

Pro Val Glu Lys Leu Asp Gly Ser Cys Arg Thr Phe Tyr Gly His Ser
      370   375   380

Arg Ala Pro Ser Ile His Leu Gln Ile Gly Ala Leu Leu Ala Ser Leu

Ala Ser Leu Val Phe Ser Leu His Pro Leu
                405   410

<210> 451
<211> 410
<212> PRT
<213> Mus musculus

<400> 451
Met Val Ile Ile Trp Trp Phe Trp Ser Leu Leu Ala Ile Cys Ala Ser
  1   5   10   15

Asp Ser Phe Arg Asp Gln Ala Val Ala Ile Met Arg Thr Thr Pro Val
               20   25   30

Ile Asp Gly His Asn Asp Leu Pro Trp Gln Leu Leu Asn Leu Phe Asn
          35   40   45

Asn Gln Leu Leu Arg Pro Asp Ala Asp Leu Asn Lys Leu Ala Gln Thr
     50   55   60

His Thr Asn Ile Pro Lys Leu Lys Ala Gly Phe Val Gly Gly Gln Phe
 65   70   75   80

Trp Ser Ala Tyr Met Pro Cys Asp Thr Gln Asn Lys Asp Ala Val Lys
               85   90   95

Arg Thr Leu Glu Gln Met Asp Val Ile His Arg Met Cys Gln Leu Tyr
              100   105   110

Pro Glu Thr Phe Met Cys Val Thr Asn Ser Ser Asp Ile Leu Gln Ala
          115   120   125

Phe Arg Arg Gly Lys Val Ala Ser Leu Ile Gly Val Glu Gly Gly His
     130   135   140

Leu Ile Asp Ser Ser Leu Gly Val Leu Arg Thr Leu Tyr His Leu Gly
145   150   155   160

Met Arg Tyr Leu Thr Leu Thr His Asn Cys Asn Thr Pro Trp Ala Asp
               165   170   175

Asn Trp Leu Val Asp Arg Gly Asp Asp Glu Ala Glu Ser His Gly Leu
          180   185   190

131

```
Ser Pro Phe Gly Lys Arg Leu Leu Asn Glu Met Thr Arg Leu Gly Val
        195                 200                 205

Met Ile Asp Leu Ser His Val Ser Val Ala Thr Met Lys Asp Ala Leu
    210                 215                 220

Gln Ile Ser Arg Ala Pro Val Ile Phe Ser His Ser Ser Ala Tyr Ser
225                 230                 235                 240

Leu Cys Pro His Arg Arg Asn Val Pro Asp Asp Val Leu Gln Leu Val
            245                 250                 255

Lys Asn Thr Ser Ser Leu Val Met Val Asn Phe Phe Ser Asn Phe Val
            260                 265                 270

Ser Cys Ser Asp Ser Ala Thr Leu Pro Gln Val Ala Asp His Leu Asp
        275                 280                 285

His Ile Lys Lys Val Ala Gly Ala Gly Ala Val Gly Leu Gly Gly Asp
    290                 295                 300

Tyr Asp Gly Val Thr Met Leu Pro Val Gly Leu Glu Asp Val Ser Lys
305                 310                 315                 320

Tyr Pro Asp Leu Ile Ala Glu Leu Leu Arg Arg Asn Trp Thr Glu Thr
            325                 330                 335

Glu Val Arg Gly Leu Leu Ala Asp Asn Leu Ile Arg Val Phe Ser Glu
            340                 345                 350

Val Glu Leu Val Ser Ser Asn Met Gln Ser Pro Glu Glu Val Pro Ile
        355                 360                 365

Thr Leu Lys Glu Leu Asp Gly Ser Cys Arg Thr Tyr Tyr Gly Tyr Ser
    370                 375                 380

Gln Ala His Ser Ile His Leu Gln Thr Gly Ala Leu Val Ala Ser Leu
385                 390                 395                 400

Ala Ser Leu Leu Phe Arg Leu His Leu Leu
            405                 410


<210> 452
<211> 410
<212> PRT
<213> Oryctolagus cuniculus
```

132

<400> 452

```
Met Trp Thr Ser Trp Trp Leu Trp Pro Leu Val Ala Val Cys Thr Ala
 1               5                  10                  15

Asp Ser Phe Leu Asp Gln Ala Gln Ile Leu Arg Val Thr Pro Val
            20              25                  30

Ile Asp Gly His Asn Asp Leu Pro Trp Gln Leu Leu Asn Lys Phe Asn
        35                  40                  45

Asn Arg Leu Gln Asp Ser Arg Ala Asn Leu Thr Val Leu Ala Asp Thr
    50                  55                  60

His Thr Asn Ile Pro Lys Leu Arg Ala Gly Phe Val Gly Gly Gln Phe
65                  70                  75                  80

Trp Ser Ala Tyr Thr Pro Cys Asp Thr Gln Asn Lys Asp Thr Val Arg
                85                  90                  95

Arg Thr Leu Glu Gln Met Asp Val Val His Arg Met Cys Gln Leu Tyr
            100                 105                 110

Pro Glu Thr Phe Leu Cys Val Thr Asp Ser Ala Gly Ile Gln Gln Ala
        115                 120                 125

Phe Arg Glu Gly Lys Val Ala Ser Leu Ile Gly Val Glu Gly Gly His
    130                 135                 140

Ser Ile Asp Ser Ser Leu Gly Val Leu Arg Ala Leu Tyr Arg Leu Gly
145                 150                 155                 160

Met Arg Tyr Leu Thr Leu Thr His Asn Cys Asn Thr Pro Trp Ala Asp
                165                 170                 175

Asn Trp Leu Val Asp Arg Gly Asp Asp Glu Ala Gln Ser Gly Gly Leu
                180                 185                 190

Ser Val Phe Gly Gln Arg Val Val Arg Glu Met Asn Arg Leu Gly Val
            195                 200                 205

Met Ile Asp Leu Ala His Val Ser Val Ala Thr Met Lys Ala Ala Leu
    210                 215                 220

Gln Leu Ser Thr Ala Pro Val Ile Phe Ser His Ser Ser Ala Phe Thr
225                 230                 235                 240

Val Cys Ala His Lys Arg Asn Val Pro Asp Asp Val Leu Gln Leu Val
                245                 250                 255
```

133

```
Lys Glu Thr Gly Ser Leu Val Met Val Asn Phe Tyr Asn Asp Tyr Val
            260                 265                 270

Ser Cys Ala Ser Glu Ala Thr Leu Ser Gln Val Ala Asp His Leu Asp
        275                 280                 285

Tyr Ile Lys Asn Val Ala Gly Ala Ala Ala Val Arg Phe Gly Gly Asp
    290                 295                 300

Phe Asp Gly Val Thr Arg Leu Pro Val Gly Leu Glu Asp Val Ser Lys
305                 310                 315                 320

Tyr Pro Asp Leu Val Ala Glu Leu Leu Arg Arg Gly Trp Thr Glu Ala
            325                 330                 335

Glu Val Arg Gly Ala Leu Ala Glu Asn Leu Leu Arg Val Phe Arg Glu
            340                 345                 350

Val Glu Gln Val Ser Asn Gln Ala Gln Val Pro Glu Glu Glu Pro Ile
        355                 360                 365

Ser Leu Glu Gln Leu Gly Gly Ser Cys Arg Thr Gln Tyr Gly Tyr Ser
    370                 375                 380

Glu Ala Pro Ser Leu His Arg Arg Pro Gly Ala Leu Leu Ala Ser Leu
385                 390                 395                 400

Ser Leu Leu Leu Leu Ser Leu Gly Leu Leu
            405                 410
```